GINA Star 4 User Manual 4.08

Content GINA Star User Manual

Measurement....3
Method....3
Start of Single Run....12
Sample table....12
General hints....13
Evaluation....16
Catalog....17
Manual Integration....22
Report....31
Calibration Using Internal and External Standards....37
Support and Version Management....47
Security and Control....50
Setup GINA Star....58
Program Installation....58
Setup GINA Star....62
Using fraction collector....68
Maintenance status....70
GINA - STAR Data Acquisition Box....71

Measurement

Method

Method parameters define the progress of the measurement and the behavior of the instruments during the run.

- From Menu Measurement / Method

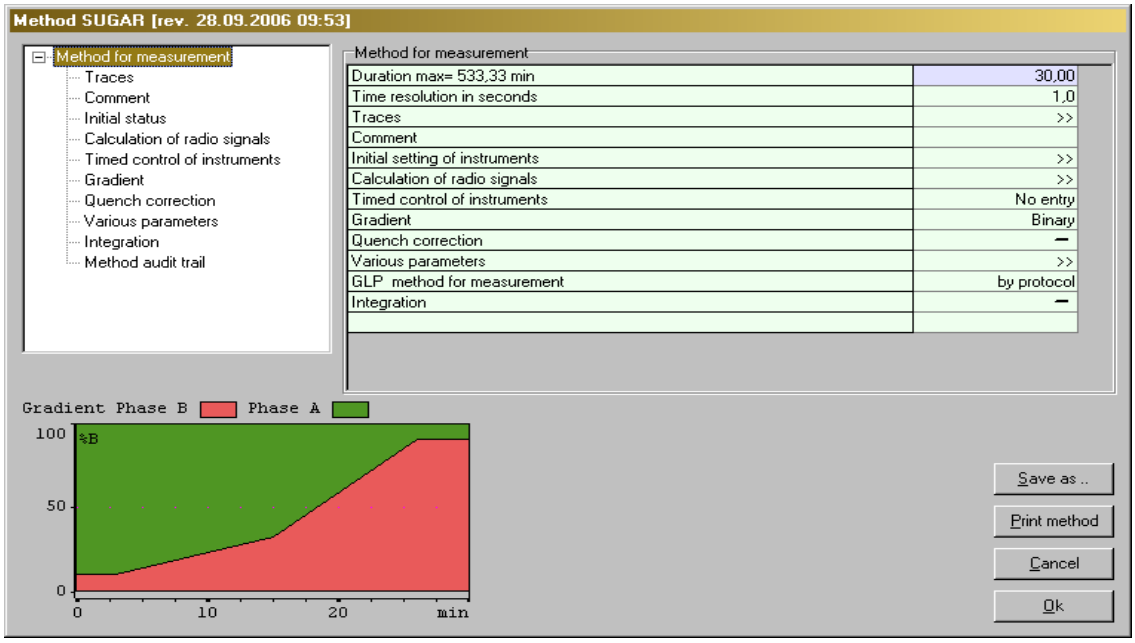


Figure 1 Method for measurement

Parameters in method for measurement

Duration	Duration of the chromatogram. During this time, signals are recorded by GINA Star as desired. Control of instruments may happen outside this time.
Time resolution	Select the time resolution required for your run. This is dependent upon the generally expected peak width. Keep in mind: each peak should be presented by at least five data points. For example, if you expect a peak width of 20 seconds, you should set the time resolution to maximum 20 seconds / 5 = 4 seconds. This field accepts entries in seconds.
Traces	Select the traces to be measured. Names, units, scaling factors and zero

	values (offset values) are reflected here from the Setup.
Comment on the Method	Each method can be provided with a comment. This comment will be copied to each measurement. Opens a editor window
Sample preparation	This menu item appears only when the autosampler is controlled by the program and offers the option to perform sample preparation / dilution.
Initial Settings of Instruments	This leads to a table to define the initial . This is also important for the start method.
Calculation of radio signals	Leads to the table **Calculation of radio signals**
Timed control of instruments	Shows the number of steps programmed. A double click leads to the table **Timed Control of Instruments**
Fraction collector control	A switch to activate or deactivate the fraction collector control. Please use the menu on the left side to enter the peak parameters.
Quench correction	Enters the table of coefficients. See Quench Correction for details.
Various parameters	Some additional parameters like periodic saving of data during run

Additional hints concerning Run Time

The maximum duration of the chromatogram which is possible with the preselected time resolution is displayed on the screen. This is dependent on the defined time resolution. Up to 32000 data points per measurement trace may be stored.
If you stop your chromatogram runs using an external stop signal, the duration set here should exceed the actual duration.

Trace parameters

Source	Physical name of the source, i.g. ADHV-3 for counter 3 on the GABI ADHV-interface board.
Name	Free name for the trace coming from the setup.
Record	Check this to record this trace in the run file.

Display	Check this to display this trace during run.
Unit	Units are fixed in the instrument or in the setup and reflected here for documentation only.
Offset	
Factor	Reflected from the setup here, see Setup Factor.
Shift	Enter here the time needed for the eluent to get from one detector to another. The other instrument (time base) should have an offset of 0.
Half l.	Only available, if 'profi' is selected. Enter the half life time of the isotope measured in this trace. Correction is not done for the background (reflected as offset above). More comfortable and more reliable
Smooth	Enter a time (or width) to define a smooth area. The smooth algorithm is selected in the setup.

Initial settings of Instrument

Here you define the conditions or initial conditions for a run. Wavelength of the UV detectors, gradient, oven temperature, etc. The contents of this menu are dependent of the connected instruments and some settings.

Calculation of radio signals (optional)

This rarely used calculation allows to calculate the DPM results from some parameters. You can define a separate set of parameters for each method. These values affect the dynamic DPM calculation.

Please notice: as long as this feature calculates a new view of the signals even during run, but in the computer and not in the instrument, it is regarded as not complaining with GMP and does not work with digital signatures. The may be subject to future changes.

For DPM, DPS or Becquerel calculation the following formula is used:

$$DPMfactor = \frac{(split * eluateFlow + scintillatorFlow)}{(cellVolume * split * efficiency)}$$

Spillover	When measuring dual labels, i.e. when using two labeled isotopes of

	different energy, there is always a “spillover” between both traces. “Spillover” is due to the nature of the energy distribution of Beta particles. Certain decay events of the higher energetic isotope are always detected in the lower energetic counting channel. Depending on the setting of the detector, the reverse case is also possible, i.e. decays of the lower energetic isotope are detected in the higher energetic counting channel. If only the spillover in one direction should be taken into account, enter ‘0’ for the irrelevant direction. If you are not satisfied with the result of the spillover correction, you can repeat the spillover correction using negative figures.
Background	Enter the count rate in cps (counts per second) as background, supplied by your detector with measurement cell, but without sample injected. When you determine this value, please note that the DPM/DPS/Becquerel calculation is turned off (set “Calculations” in the last row of this menu to “No”). The entered value appears in the table “Trace parameters” in the column “Background”, if necessary, transformed to DPM or DPS.
Scintillator flow	This information is required for “dynamic flow control” (see Calculation below). The entered value is used only for calculation, it does not control the actual scintillator flow. The control of the scintillator flow takes place via the menus and Timed control of instruments.
Eluate flow	Enter the eluate flow in ml/min. The entered value does not affect the actual eluate flow, but it is required for “Dynamic DPM calculation” (see Calculation below).
Cell volume	Enter the volume of your cell in µl or ml. The volume of the cell is generally printed on the cell body. This value is used for “dynamic flow control” (see Calculation below).
Split	f you split off part of the eluate before adding scintillator using a splitter, enter the percentage of the eluate that flows into the measuring cell. This value is required for “Dynamic flow control” (see Calculation below).
Efficiency	Enter the statistical efficiency in percent. Please take this value from the label on the measurement cell. If there is no label on the cell, the

	statistical efficiency has to be determined through measurement.
Calculation	Here you can select in which units the results of the measurement should be calculated. Choose between DPM, DPS, Becquerel or raw data output.

Please note, that the instrument must not perform any correction, if you use this calculation!

Timed Control of Instruments

In this menu you can set up a list of timed events. Connected instruments can be controlled according to this list. For example, it is possible to change the wavelength of a connected detector during run at a predefined time. The controls appearing here dependent on your system configuration.

Timed control table

Time	Enter the time for the desired setting. The time is calculated from the start of the measurement (generally identical with the time of the injection). '+' or '-' time shifts actual entry and the rest of the table at once. For new entries to the time table, enter time relative to the previous entry.
Duration	This column gives a second view to the time information. It represents the time to the next event. You may simply enter a new duration here instead of + absolute time in the time column.
Control	Move the cursor into this column and push the space bar or double click; this opens a window that includes all controllable parameters. Using the cursor keys, select one and push Enter.
Value	Enter the value for the parameter to be controlled, i.e. select 'open' or 'closed' for a 2/2 way valve.
Hint	Type a comment here. The comment will appear on the instrument control screen during the run and should help you to keep orientation during along a complex control

Keys available in the Timed Control of Instruments

<Ins>	Inserts a new line to the time table
<Del>	Deletes a line from the time table
<F8>	Copies the time table to the clipboard, valid for every table

<F9>	Prints the time table (not the whole method)

Items to change within the column "Control"

The column "Control" allows to change all items available in the "Initial status", thus valves, UV-wavelength etc.. There are some further entries to control the execution of this table itself:

Wait for event and **Wait for -event**	During run time these entries allow to pause the execution of the time table until the state of the selected event is true or false (wait for -event). Because the time is unpredictable, the time column restarts with the time "tx+"
Segment	This is a headline for a chapter. You may enter free text to the column "Control" to structure you time table and keep long tables readable.
Timed control	With the value "hold" this entry causes the execution to wait until a user enters a "continue" command (F2 in the actual time table)
Raise error *)	This causes a run time error, the hint is used for the error message. This entry may be used with conditional jumps to notice special situations.
Label	This entry defines a
If event go to *)	This entry jumps to a label number, if the state of the selected event is true.
If -event go to *)	This entry jumps to a label number, if the state of the selected event is false.

*) these rarely used entries are available, if the text "profi" is entered in Setup / License / License addendum or 'Setup / Extra / Expanded time table' is checked

While running the measurement and viewing the recorder trays, you may view this table proceeding by typing CTRL-Y for the actual time table.

Special Functions

The special functions described below are not, or only rarely, needed during regular operation.

Ctrl-S: Manual fraction	Starts the integration area and advises the fraction collector to fill a new vial. (This command is available from the instrument control screen as well)
Ctrl-T: Manual fraction	Stops filling the current vial, Marks the peak end and indexes the fraction collector to the next vial. (This command is available from the instrument control screen as well)

Ctrl-S: Manual fraction	Starts the integration area and advises the fraction collector to fill a new vial. (This command is available from the instrument control screen as well)
Ctrl-Y: Actual list of timed control	After a measurement has been started, you may watch the progress of the timed control of events. Here the following commands are available: <F2> hold or continue depending on the state <F3> execute the next step, ignore the programmed wait time <F5> skip the next step (i.e. a wait event) and continue (This command is available from the instrument control screen as well)
F4 Start	This commands allows to manually start a measurement. It is available in the measurement view and only if GINA Star controls no auto sampler. (This command is available from the instrument control screen as well)

Handling of 'Hold' during execution of timed control

The programmed hold is used to wait for some user actions to take place. After that, the user re-enables the execution by resetting to the 'Run' state. Please use the hint to inform the user what to do, especially if more than one 'hold' is within the list. GINA Star does the following actions (Vers. 4.04 ff):

- Execution of time table is paused
- Instrument control page is opened, if 'Timed control' is not represented on the graphic view, the text mode is used.
- The message from the hint column is displayed and the user may double click to 'Hold' to enter the run mode again.

Gradient

The table of gradient steps opens along with a graphic representation if the gradient.

Time	Enter here the time for a change of the gradient. The value “Initial” in the first row cannot be changed, because in this row you set the initial conditions of the run.
%A, %B, %C	Phase B and C can be freely selected in percent. Phase A is calculated by the computer: %Phase A = 100 - %Phase B - %Phase C
Flow	Enter the total flow in milliliters per minute.
Type	Non-linear gradient shape for the respective gradient step. Select between 1 and 11. Values between 1 and 5 create convex, values between 7 and 11 concave curves. For a linear curve enter “0” or a hyphen.

Fraction collector control

If the program includes the feature Fraction collector control, you can set the desired peak search parameters in the dialog box Fraction collector control.

Search mode	Select a peak search mode from the drop down menu. You may choose: *Shape:* peak search based on a mathematical function which evaluates the curvature and the change of curvature from the measured curve *Level:* peak search based on the height of the measured signals. If the entered level is exceeded, the substance is collected in a vial. A waste signal is available in this mode at the terminal “Aux out”. *Manual:* Cut the peaks by pressing CTRL-S (start) and CTRL-T (terminate) during run
Peak reject	If search mode 'shape': peak reject indicates how high the contents of a peak has to be at least in order not to be rejected. If the peak search program integrates pseudo peaks in the background noise of a chromatogram, this parameter should be increased. The permitted range for peak reject extends from any small number (no peak found is rejected) up to any high number.
Peak width	Enter an approximate peak width
Vial fill time	Necessary if no waste signal is used. In this case, GINA Star will proceed to the next vial, if one is filled by the waste
Cell to vial delay	Generats a delay for switching the collector to represent the time used to pass the outlet capillary
Trace	Select the trace in which the peak search is to be performed from the drop down list
Timed events	Leads to the table of timed events listed for the fraction collector
Switch on levelSwitch off level	The switch on level defines the input signal for start of the fractioning process. If the signal drops below the switch off level, the system switches back to “Waste”. When you enter the switch on level, the program sets the switch off level to a value that is 10 % lower than this level.Is the switch on level is lower than the switch off level, the program scans for negative peaks.

Various parameters

Here you find some parameters of the measurement method more rarely used.

Equilibration time	Enter the equilibration time, which should elapse before getting ready for start prior to each measuremnt started with this method.
Autosave	Apply a time here. GINA Star will save the current measurement everytime, this times elapses to protect your data against power failure or crash.
Archive after measurment	Copy the data to the archive at the end of a measurment
Dead-time of column	Enter the dead-time of the column. This unit does not have any influence on the measurement. However, the dead-time of the column is required in the integration program to calculate the capacity factor and the selectivity. See chapters and .
Common decay time	Half life time for postrun correction. The correction is done for the 'Corrected region area' only.

GLP method

To help you meet the requirements of GLP (Good Laboratory Practice), we have introduced the GLP attribute.

The meaning of this parameter has changed to GINA Star version 4.01. Methods are no longer protected against changes. You may now process evaluations on measurements measured with this option 'strict' (a raw data file is always protected by GINA Star). The only effect of this option is, that the measurement is processed without any influence of the user.

GLP_normal	Runs with this method are fully controlled as configured in the access control.
GLP_strict	In addition to normal, runs with this method must not be affected by manual instrument control.
GLP_unregulated	If access control is established, runs with this method are not controlled, no audit trails, security copies etc are generated and the run may later be deleted.

Integration after each measurement

GINA Star *performs a automatic integration at the end of each run according to the rules defined in the Integration method.*

To enable the integration for the end of each run, please select the item by toggling with <Spacebar>. The <F2>-key allows to edit the corresponding Integration method.

Start of Single Run

This optional item is available only if no autosampler is configured.

- From menu select Measurement / Start single measurement

Click icon 'Start single'

If you are working with an injection valve, you start measurements without the sample table. Enter simply the name of the measurement, if necessary, select a method from the list and start. You should enter a comment to describe the sample.

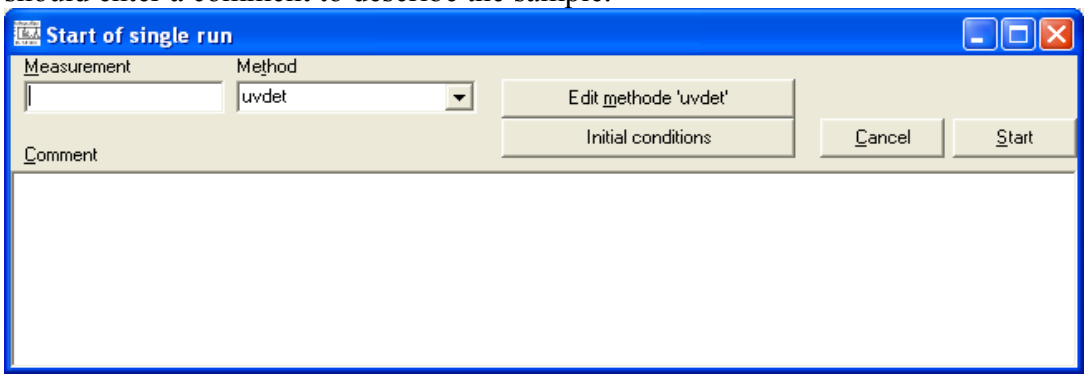


Figure 2: Start of single run

Measurement	This is the run name and must be at the same time a legal file name.
Method	Select a method from the drop down list. See compatibility for opening methods from GINA 2.18 and 3.00.
Edit method	This is a last opportunity to check or to modify the selected method.
Initial conditions	Normally the initial conditions of the selected method will be established in the instruments as soon as you press 'Start'. If you want to do this before or you intend to only set these initial conditions, press this button.
Comment	Some lines of text will be saved in the measurement and printed along with the report
Study (optional)	Studies are selected, you have a drop down field here to select a study for this run.

Sample table

To start a measurement or a series of measurements, you have to create a entries to the sample table.

- From menu select Measurement / Sample table or
- Click the icon 'Sample table'

Figure 1 Sample table

Content GINA Star Sample Table

Measurement....3
Method....3
Parameters in method for measurement....3
Additional hints concerning Run Time....4
Trace parameters....4
Initial settings of Instrument....5
Calculation of radio signals (optional)....5
Timed Control of Instruments....7
Keys available in the Timed Control of Instruments....7
Items to change within the column “Control”....8
Special Functions....8
Handling of 'Hold' during execution of timed control....9
Fraction collector control....10
Various parameters....10
GLP method....11
Integration after each measurement....11
Start of Single Run....12
Sample table....12
General hints....13
Entries in the sample table....14
Import sample table from foreign programs....15
Continue / Pause Processing of Sample Table....16

General hints

You may insert the measurement anywhere below the dark gray area. The dark gray area contains the entries already measured. Using the <Ins> button you can insert a measurement between two entries. A preselected measurement can be removed again by pushing the <Del> button.
Already measured entries can be called by double-clicking the name.
If you have quit the list again by pushing <Esc>, and you have confirmed the question concerning start of processing the sample table, you may push <F4> to start the first measurement.
Measured runs and runs that are currently active appear in the sample table only if you have not selected automatic deletion in . If they appear, you can call the measured data set with a double-

click.
You may export sample tables to the clipboard by help of the copy icon.

Entries in the sample table

Study (optional)	Enter to which study the respective measurement is to be assigned. This information helps to organize and track measured data and does not have any effect on the measurement process. The second section of the search path contains the name of the instrument and is defined in the settings. Enable this optional field in Setup / Path.
Method	Select the method for your run either from drop down list or by entring a name. You may edit the selected method by typing <F2> or pressing the 'Edit' button
Measurement name	Select a name for your run. The name may comprise up to 29 characters. You may use an extension. This name is an unique identifier for the run and will always be printed in the report independently of the filename. To enter a series of runs just add the index range i.g. m.3-10 for m.03, m.04 ....
Position	This column is important only when your autosampler is controlled by the PC and has free access to the samples. In this case, enter the position of the respective sample in your autosampler.
Injection volume	The entered value is stored for documentation and calculation in the data set. If your autosampler is connected to a PC, the injection volume is transferred to the autosampler.
Weight	Enter the actual weight for sample weight correction. Weight correction makes it easier to weigh your samples. The target value need not be reached exactly. In the respective column of the sample table, enter the actually weighed sample volume. The program automatically corrects for the difference. This column appears only when weight correction has been enabled in *Setup* |
Dilution	The entered value is stored in the data set for documentation and calculation. If your autosampler is connected to a PC, the dilution is transferred to the autosampler.
Sample type	Enter the sample type. It can either be an external standard (Std1 to Std16) or an unknown sample. For the standards, enter “1” to “16” and for a sample to be measured “0” (zero). Each standard number belongs to a column in the list “Components of standards”.

Number of internal standards	Enter the concentration of the internal standard in the unit you have preselected in the menu .
Speed	This menu item appears only for certain types of autosamplers. Sample draw and injection speed.
Status	In this column you can view the progress of processing the sample table. Already measured samples are marked with time and data of begin of run. Samples that have yet to be measured are labeled “wait”. Push “+” on the numerical keypad to move the marker down, indicating which sample is the be measured next; push “-“ on the numerical keypad to move the marker up. If you enter a period (“.”), the bar will jump to the row containing the cursor
Comment	For each individual run you may enter a comment here. Move the cursor to the *Comment* column. Push the <spacebar>. A window below the table opens. In this windows you can edit the comment. All functions of a standard editor are available, including copy and paste.

Import sample table from foreign programs

GINA - STAR is able to accept sample tables made by foreign software. The format is TAB separated text. The columns have meanings defined by keywords in the headline.

STUDY	Study name, used only of 'Setup / user options / support studies' is selected
METHOD	method name
SAMPLE	measurement name
POS	sample position number in autosampler
VOL	sample volume in µl
WEIGHT	sample weight before injection
DILUT	dilution of sample
ISTD	amount of internal standard 1
DRAWSPEED	sample draw and push speed in µl/min
NEEDLE	needle hub for HP autosamplers
DOCVOL	defines output path for documentation files *)
INSTRUMEN	if present, the value is checked to be equal to 'Setup / user options /

STUDY	Study name, used only of 'Setup / user options / support studies' is selected
T	instrument's directory'
COMMENT1	comment line 1
COMMENT2	comment line 2
.....	.....
COMMENT9	comment line 9

*) DOCVOL is treated as a complete file name, if it contains a '.' in the last name segment. I.g. '\\labserver1\fstudyx\nSampleABC.TXT' will be used as file name for the report. If there is no dot in the last file segment (after the last '\'), GINA - STAR will interpret the name as a directory name and will add the name of the measurement plus '.TXT'. If the name ends with '\' , the normal path segement for study, instrument and method is added (or only method, if 'use studies' is not used).
If you want to have TABs within a comment, you enclose the comment in double hyphens "comment TAB comment"
The columns may be in any order. The column headers are key words and are read case insensitive. Not all columns need to be present. Of course the column 'Sample' must be present. Other missing columns have the same default as if they were typed to the list.
This list has to have the file suffix '.LST' and may be opened by help of GINA - STAR's browser or the file may be dragged and dropped over GINA - STAR's icon or GINA - STAR's program window.

Continue / Pause Processing of Sample Table

- From menu select Measurement / Pause execution of sample table
- From menu select Measurement / Sample table continue

This does not affect the current run. Only the start of a next sample is delayed, if the execution of the sample table is paused.
With the sample table the recorder buttons like play show the status. From the menu Measurement, the selection symbols show the status of the sample table.
Please note: after starting GINA Star, the sample table is always paused.

Evaluation

Evaluation and data handling

Evaluation....16
Catalog....17

Catalog Items....19
Buttons....21
Context menus....22
Drag and Drop within the catalog....22
Manual Integration....22
Define Integration Regions for Radioactivity Channels....23
Define Integration Regions for Analog Channels....23
Groups of regions and baseline....24
Commands for Manual Integration....24
Special Functions....27
Information....27
Evaluation of radioactivity....28
Count rates and statistics....28
Units and integration....29
Signals and background....29
Samples and Total activity....29
Estimate the limit of detection....30
Background subtraction and 'negative'....30
Report....31
Sample description....31
Preparation....32
Manual control audit trail....32
Measurement audit trail....32
Integration....32
Report Design and Report Columns....32
Report generator....32
Footer lines....35
Remainder (optional)....35
Total (only radio traces)....35
BKG....35
Peak search....36
Items to specifically influence the peak search....36

Catalog

The file handling functions deal with files containing measurement runs. In addition to open these files for evaluation, this dialog allows to execute more function i.g. from various context menus for single files or any selected collection.

- From menu ' File / Open' or 'File / Archive' to view and open from archive
- Icon from icon bar

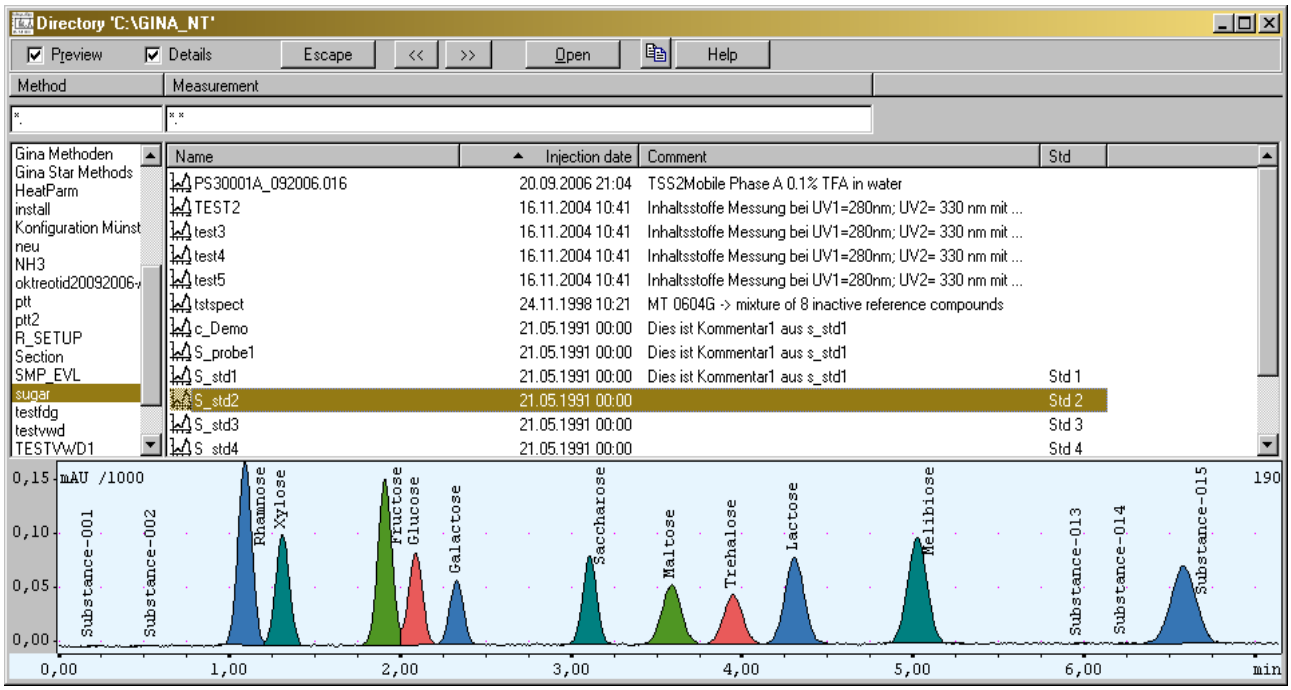


Figure 2 Directory with preview

The main task of this menu is to open files for evaluation and for generation of reports. Files may be opened by a double click or by pushing the open button.

The default catalog view contains the preview of the chromatrogram and an icon in front of the name. These icons have the following meaning:

	Normal run. RAW data are secured and guaranteed to be unaffected.
	This icon indicates signature not present in the data file. This may happen, if data are imported from ancient GINA Star versions 2.18 or 3.00 or from other software not supporting signatures.
	The signature became invalid due to any impact to the RAW data. No approval may be applied to evaluations.
	This run is evaluated and the evaluation is marked 'completed'.
	This run is approved, what means the evaluation is completed and this has been approved. All information is secured.

	This icon is used temporarily to indicate catalog entries with signature not yet checked. Wait some seconds and this icon will be replaced.

Catalog Items

The catalog may be invoked for the work path (also by help of the icon) or for the archive. The catalog offers a lot of functions via the context menus in addition to the buttons.

Preview Uses one part of the window to show the content the selected file

Details Click this button to view the name of the data set, the injection time and the first comment row. At the same time, all information, except the comment, can be used as sort criterion. To sort, simply click on the respective column header. Since the information cannot be stored in the catalog entry of the file system, the respective data set has to be opened for each entry, so that the screen builds up a little bit slower. On network drives, this may slow down catalog operations dramatically.

Study If you have enabled *Support studies in the setup / Print Options* the *Study* column a filter edit appears in the 'Open' dialog.

Instrument If you have enabled *Support studies* in Print Options, the *Instrument* column appears in the 'Open' dialog. The presetting is '*', i.e. the data sets of all instruments are listed.

Method The default setting is '*', so that all methods are listed for which a data set has been created. You may enter any restriction Windows permits, e.g. 'FDG*' lists all methods starting with FDG. Simply click on the method within which you are searching for the data sets.

Selecting data Every list has an edit field at the top to filter the data in the corresponding list. The default value is '*.*' to list all data. You may enter any Windows-conform restriction, e.g. 'x*.*' for all data sets starting with the character 'x'.

Select one or several data sets. Hold down the Shift button to select entire ranges of data sets, hold down the Ctrl button to select individual data sets or unselect them again.

Deleting data sets	First, select all data sets you want to delete and push <DEL> or select ‘Delete’ from the context menu.
Editing names and comment	Not yet implemented. Instead, load the data set, rename it, possibly edit the comment, and save it.
Archiving data sets	Select the desired data sets and select ‘Archive’ in the context menu or push <CTRL-A>. If you are in the archive dialog, this command is not available. However, you can open the data sets directly from the archive.
Removing a method	You can delete methods from the ‘Open’ menu. Push <Del> or select ‘Delete’ in the context menu. If the subdirectory of this method still includes data sets, the message ‘Method directory not empty’ appears and the method cannot be deleted.
Removing a study	Select a study and push <Del> or select 'delete' from the context menu.
Copying to another method	To copy data sets to another method, drag the selection in the method window to the desired method. The method to which you want to copy is identified by a dotted line.

Buttons

The catalog shows the measurements and allows you to select any number of files. The selection is done by the standard windows procedure. Thus you select the first file, hold the shift key and select the last file. Or you select the first file, press the CTRL – key and select successive files.

As shown in Figure 2 Directory with preview , some buttons are available in the catalog:

	Copy the catalog to the clipboard. Creates a tab separated text representing the selected part of the catalog. If no files are explicitly selected, the whole catalog is transferred
Open	Open the measurement for evaluation. This may alternatively be done by a double click to the file.
Preview	The preview allows to view the selected chromatogram prior to loading it. At the same time the number of files in the catalog is reduced to make space for the preview.
Details	Selecting details shows some extra columns in the catalog containing information from the file: injection date and time, first comment line and sample type. The makes it necessary to open the file and slows down catalog speed dramatically in network directories.
<< | >>	Two navigation buttons
Escape	Escapes the catalog without further action

The catalog allows you to drag files from one method or study and drop it to one another. Furthermore each list of the catalog (measurement files, methods or studies) have a context menu to be called by clicking with the right mouse button

Context menus

Each list maintains a context menu to offer more functions. The file list has the following functions

Rename	Rename the file
Delete	Delete the selected files
Archive	Copy the selected files to the archive. The archive is defined in the Setup / Path
Print report	Print the report of all selected files according to the documentation parameters in the integration method.
Execute evaluation	Execute the evaluation of all selected files according to the integration parameters in the integration method.
Select evaluation method	GINA Star selects a evaluation method with the name of the measurement's method by default. Here you may select a different one.
Sign evaluations (optional with user management)	This applies the approval to the selected measurements.

Drag and Drop within the catalog

Drag and drop is provided to move measurements from one method to another or from one study to another. This move does not affect the information about the method or study in the report nor does this affect the used method for evaluation. Use the context menu to change the evaluation method for a group of measurements. The information about the used measurement method cannot be changed.

Manual Integration

The program part Manual Integration allows you to view and (re-)integrate chromatograms, and also, if needed, to perform a fine tuning after automatic evaluation. Here you find:

Define Integration Regions for Radioactivity Channels

Background regions

Select the button for background regions . Push this button and drag the mouse across a certain chromatogram area to create a background region. You can then grab and drag the margin delimiter. You can delete background regions by moving the right delimiter to the left delimiter. When all regions have been set, unselect the button for the background regions again by clicking another button, preferable the zoom button

Individual regions

Select the button 'Drop-Regions' . If this button is pushed, you can create 'Drop' regions and separate existing ones. Simply drag the mouse across the region you want to integrate. When all regions have been set, unselect the button for the background regions again by clicking another button, preferable the zoom button .Groups of peaks

Region groups

Select the button 'Drop-Regions' . Push this button and you can drag the mouse across an entire group of adjacent regions. Then you separate them by clicking in the valleys one by one. Without making a new selection, you can then start the next group. When all regions have been set, unselect the button for the background regions again by clicking another button, preferable the zoom button

Define Integration Regions for Analog Channels

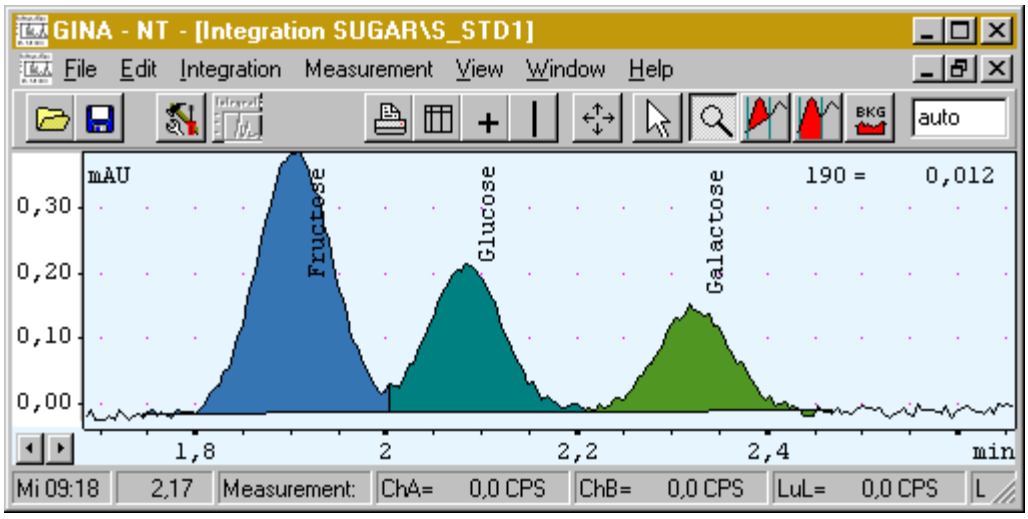


Figure 3: Region group

Individual regions	Select the button for baseline separated regions . Push this button to create regions. Simply drag the mouse across the region you want to integrate. When all regions have been set, unselect the button for the background regions again by clicking another button, preferable the zoom button .
Groups of regions	Select the button for baseline separated regions . Now drag the mouse across the entire range of a group of peaks. Select 'Drop' and click in the valleys inside of the selected region. When all regions have been set, unselect the button for the background regions again by clicking another button, preferable the zoom button .
Correction of region delimiters	You can edit all features of a region subsequently. Select a picture detail, then click on a region. Now the region delimiters appear as black dotted lines around the region. If you move the mouse left or right over a line, the cursor appears allowing you to shift the delimiters . At the end you drag and drop the delimiter at the new position.

Groups of regions and baseline

In the manual integration you have a big influence on the behavior of the baseline to calculate for different effects on you own responsibility. Normally the baseline goes straight from one B to another 'B'. Some special functions are not available with the mouse but from the keyboard.

H Horizontal	Horizontal means, that the baseline goes from one 'B' parallel to the x-axis. In the case, you only have one single 'B' in the group. The 'H' separator allow to influence this behavior. The baseline will be parallel to the x-axis between the 'B' and the 'H'
T Tangential	The tangential peak separator does not affect the baseline, but 'skims' some portion of a peak to the unseparated neighbor

Commands for Manual Integration

This chapter describes the key commands available for manual integration and baseline definition. Except for the 'T' and 'H' region delimiter, these functions are as well available in the 'Edit' or context menu.

+ Invoke integration	Executes the automatic integration and identification routine according the the integration parameters.

A Smoothing	Enter a smothing grade for the selected trace in the units of the x-axis.
C Clear region	Delete a single region, if selected
B Set region delimiter	Sets a region delimiter of type B. Fixes the baseline at this point of the chromatogram. Meaningful for single peaks or as outside delimiter of a peak group
D Set region delimiter	Sets a region delimiter of type D. Perpendicular separation of two unresolved peaks. From the measurement curve, there is a perpendicular drop to the baseline. Only meaningful inside a peak group
H Set region delimiter	Rarely used horizontal forward or backward to generate horizontal baseline from this point
I Peak characteristics	Display of chromatographic characteristics in the table peak characteristics
J Jump cursor	Jump to a given x-position
K Edit comment	The comment lines, which are printed out in each report, can already be entered when entering the measurement in the sample table as well as in the methods parameters. Moreover, comments can be invoked and, if necessary, edited during manual integration. After input of the command ‘K’, the comment is displayed on the screen and can now be edited as needed.
M Control measurement	During measurement, this command
O Load original	The button ‘O’ allows you to reload the file from disk to recover from erroneous operations in the evaluation.
P Plot / report	Open the the report and allow to print the documents according to the setup.
Q Quench	Invokes the Fehler: Referenz nicht gefunden page in conjunction with the method.
R Rename region	To edit the name of an integration region, set the cursor in this region and type “R”

T Set region delimiter	Tangential separation between two unresolved peaks. Can only be applied subsequently within a peak group. Separation line can be rotated around tangent point using the command <Shift><Up> (push shift button and up arrow key at the same time) and <Shift><Down> (push shift button and down arrow key at the same time)
XT Zoom all	Zoom x-axis to display the whole area. With the button Zoom all you can display the entire chromatogram.
Y y-scaling	Type 'Y' to change the Y-scaling of the selected trace. (Y-label inverted, can be changed by <PgUp> or <PgDn>). A dialog window appears. You may just enter a single value forcing the scaling from zero to this value. If the value is zero, GINA retrurns to automatic scaling. You may furthermore enter a range 'from-to'.
V View traces	This command accepts 1 or more digits. First, enter the number of traces you want to see. Then if necessary select the traces you want to see from the list.
W Display peak labels	Select between label peak name, retention time or no label.
<ESC>	The escape key allows to leave a dialog sequence
Move region delimiter right	Command <Shift> <right> (push shift button and right arrow key at the same time): Position the cursor on an already existing region delimiter to the right of the cursor. Using the same command, you can now move the delimiter
<PgUp>, <PgDn> select trace	Selection of the active trace for multi-trace measurements. The selected trace is marked by the color behind the y-axes label. All trace specific actions like scale y-axis affect this trace.
Move region delimiter left	Command <Shift> <left> (push shift button and left arrow key at the same time): Position the cursor on an already existing region delimiter to the left of the cursor. Using the same command, you can now move the delimiter
Correct tangent slope	Command <Shift> <Up> (push shift button and up arrow key at the same time): Rotates a separation between two peaks of type T or D counter-clockwise

Change tangent slope	Command <Shift> <Down> (push shift button and down arrow key at the same time): Rotates a separation between two peaks of type T or D clockwise
Export to Word	The export function copies the graphic and all report tables to a word document. The tables are formatted as default tables. The Word editor remains open and the user is responsible to save the file.

Special Functions

The special functions described below are not, or only rarely, needed during regular operation.

Ctrl-V: Raw data	Presents the raw data of the chromatogram in a table. Data cannot be changed manually. Push <F9> to print the table of raw data. Push F8 to export the data in text format.
Ctrl-Z: Display integration regions	Displays all integration regions defined. Option to edit their name or other attributes (Name position, etc.)

Information

- From the context menu call 'Information'
- From the menu call Edit / Information

The information menu consists of a tabbed screen to display additional information. The tabs group this information into:

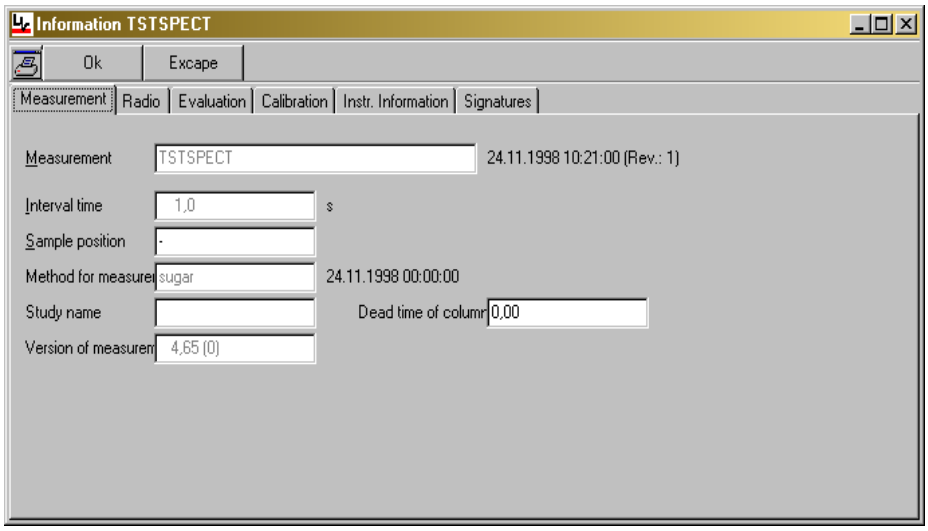


Figure 4: Information, tab Measurement
Here you find the original run name, Injection time and date, the interval time, sample position if autosampler was used, name of the method and then build number of the software running the measurement (in the above example 0, because 4.65 is a DOS software release).

Evaluation of radioactivity

Labeling of chemical and pharmaceutical substances gives answers to questions which cannot be given from standard chromatography. I. g. the question “is this substance a metabolite of the origin or only produced by some other way while the pharmaceutical is present”.

The disadvantage of radioactivity is, it owns some properties to which most scientists are not as familiar as to integration of UV-signals. Some effects appear and questions come up. These pages try to give answers.

Count rates and statistics

Radioactive decay is for itself a statistical process. This cannot be overcome by improving detectors. If a nuclide is unstable at all, from a big number of atoms half of them will have decayed after a certain time which is characteristic for each isotope (half life time). This does not predict the behavior of a specific atom, it is completely unpredictable, when this will decay. In other words, big numbers of atoms make the statical effects invisible while small numbers (low activity) will show this effect. Unfortunately, there are a lot of reasons to label substances with low amount of activity.

First, even marking 100% of the interesting source may result to low activity in the sample. Next, high activities may impact the sample itself (auto-radiolysis). Finally, radioactivity impacts the human health, thus it impacts people dealing with radioactivity. For these and other reasons, experiments are done with very low activities and therefore high statistical effects. The signal shown in the software is more like a forest than a curve. Software smoothing and calculating signals to show smooth curves like UV-signals through away important information, as the following explanations will show.

Units and integration

Every measurement tries to get a property of a material or a sample (i.g Bequerel per ML). The measurement by help of a flow cell is somewhat complicated, the obtained count rate depends on:

- The geometry. Not all particles from a decay reach a detector.
- The counting efficiency or the part of desintegrations making an event to the counter
- Cell volume or the amount of sample visible to the detector
- Flow rate makes effect to the peak area (see GINA Star user manual / Calculation of Radio Signals for more details)

The last point says: the result of the measurement is (except an efficiency factor) what activity moves through the cell per time unit or

$$\frac{(Bq\,(activity))}{s} \quad \text{or in the case of TLC} \quad \frac{(Bq\,(activity))}{mm}$$

The integration over the time (or the distance in TLC) gives back the activity of the peak. The y-scale of the graph in the chromatography is **not** a count rate nor the activity, this is obtained by the integration. Please regard: from the view of a counter, this would be wrong, but viewing the system flow cell and counter, this is correct!

Signals and background

The eluent moves components from the column to the cell. Here the radiation is detected and a higher count rate is shown. Without any component, the count rate does not become zero, but a certain count rate called “background”. This is from natural radiation coming from walls, atmosphere, ground and maybe from rests of previous measurements. This count rate should not be subtracted because it contributes to the statistical variance of the signal above this background.

Samples and Total activity

In most cases, the distribution of activity to different substances has to be determined. This distribution does not depend on the absolute scale, thus no DPM calculation is necessary for this. CPS/s may be integrated to CPS.

The activity of the sample is distributed over the chromatogram. Every peak represents a component and contributes a certain amount to the total activity. In the best case, after integration, the total activity is resolved in peaks and clarified by this way. Total activity, the sum in regions and

the remainder (difference of these two) is calculated by GINA Star. A raw rule is: the activity is resolved, if the remainder is within +/- 5% or some of regions is 100% +/- 5%. The Integration may be higher than 100% because the Total activity does not contain the background but type 'B' regions may integrate areas regarded as background by the calculation of total. Please avoid regions of type 'B' in radio – traces and regard the hints in the GINA Star manual.

Estimate the limit of detection

See also: Open File / Setup / Edit setup / User options / Dialog

If you did setup 'Peak width to display limit of detection' to the estimated width at half height of your peaks and 'Factor for sigma' to i.g. 3, the display will show two help lines, one for the background defined by the BKG – region and one which has to be touched by the region to fulfill the sigma condition.

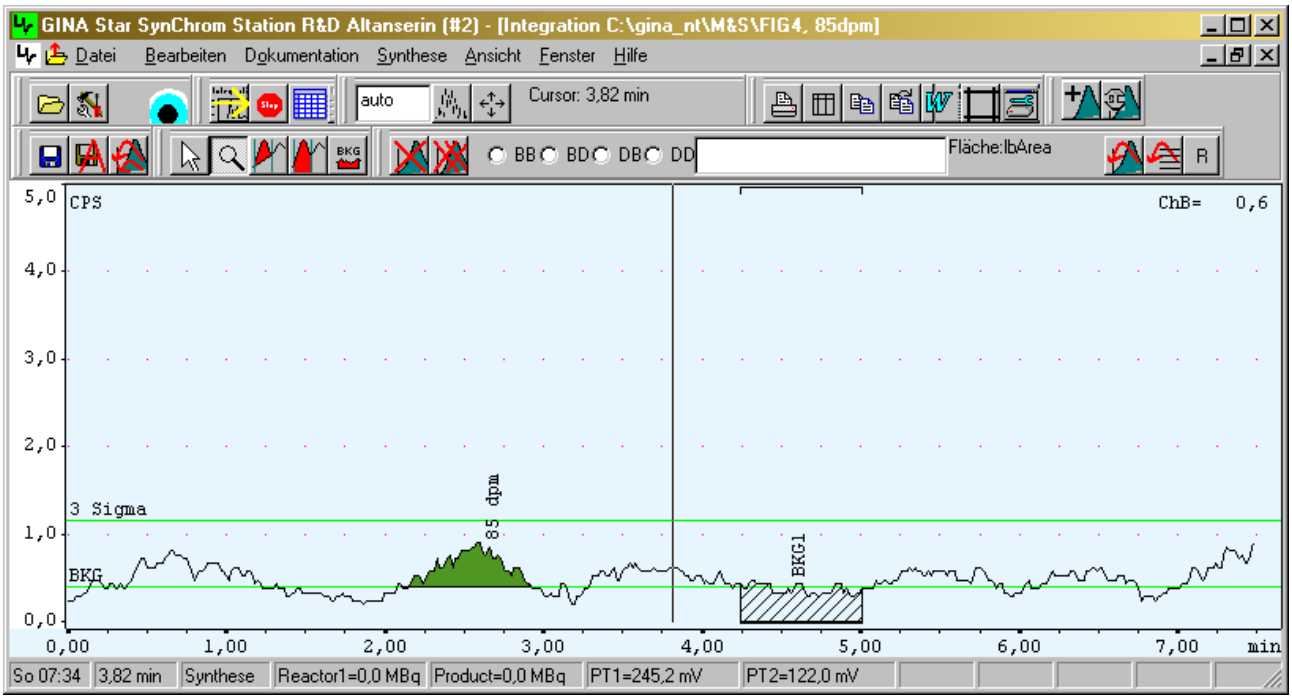


Figure 56: Estimate limit of detection

Background subtraction and 'negative'

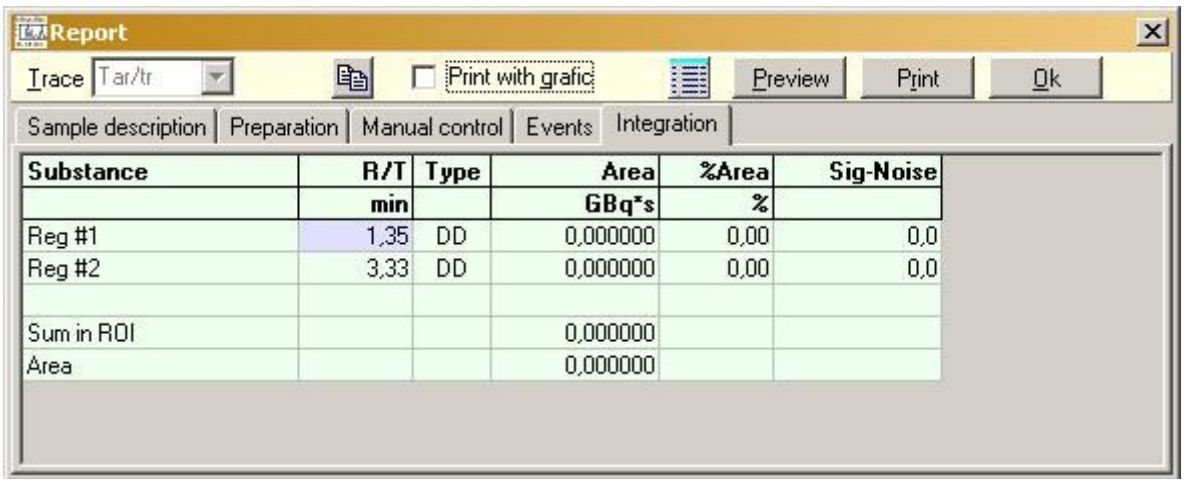
If background subtraction is even done (as like in some instruments of various supplies), a so called CPS value (net counts per second) is calculated. Some people think, that this value must not become negative ("count rates cannot be negative!") and instruments are built truncating these to zero. This gives wrong results as the following example shows: imagine a background of one CPS measured in one second time intervals. Some intervals will give zero, some 1 and some 2 rarely 3 will occur, the mean count rate over 10 minutes will be 1. To calculate this example, I use a simplified statistic resulting in 2 for half of all time intervals and 0 for the rest, 600 counts after 10 minutes. After background subtraction, the intervals with 2 result to a CPS value of 1 and from 0 a CPS rate of -1 is calculated. After 10 minutes, the sum of all "CPS" interval rates is zero as we

expect after background subtraction. If now negative CPS values are truncated to zero, only positive contributes remain and we get a sum of 300 in this simplified statistics (somewhat less than 300 in real statistics). This is more worse than only theoretically wrong, this decreases the peaks above the background, because within the peak, there is enough counts to subtract the background.

Report

The report is a collection of information provided for every measurement file by GINA Star. It may be viewed, printed and exported to files or other systems and may be invoked from many places in GINA Star:

- From manual evaluation by command 'P' (print report)
- From manual evaluation, context menu 'Print report'
- From the catalog select a collection of runs, right click and from context menu select 'Report'
- As part of an automatic integration, select 'Print report' in the evaluation method



Substance	R/T	Type	Area	%Area	Sig-Noise
	min		GBq*s	%	
Reg #1	1,35	DD	0,000000	0,00	0,0
Reg #2	3,33	DD	0,000000	0,00	0,0
Sum in ROI			0,000000		
Area			0,000000		

Figure 7 Report view

Sample description

This report section contains some general information about the sample.

Preparation

The preparation contains a sequence of user actions, needed to start a run. Here, the user may be enforced to document charge numbers from contributes, confirm the presence of tools and properties. This page is normally not used and disabled in the Setup / User options / Dialog.

Manual control audit trail

Every action applied by the user during measurement affecting the measurement itself are recorded in this trail. If the user changes the run total run time, the UV-wavelength

Measurement audit trail

This trail covers every change on any item of the measurement method including creating and copying. Thus it may be helpful instrument during method development. It is not printed along with the report because it covers information coming from the method and information reported in the 'Manual control audit trail'.

Integration

This area contains the result tables of the integration. The information appearing here depends on the selections in the report generator and on the properties of the measurement trace. Traces from radio detectors contain some additional information. Traces for quality control cannot be integrated, i.e. the pressure signal from the pump.

Report Design and Report Columns

To define the contents of a report, edit two properties:

- File Setup / Edit setup / Options / Print options
- From evaluation Report / Report generator

Report generator

Using Report generator, you can select the columns appearing in your report and design their format. Select from a defaulted list of output values the one that is relevant for you, determine under which header this value should appear and which width the respective column should have. You can also set the output format.

- From the report use the icon 'Report generator'.

- Call the integration method and select Report generator from the left hand menu.

Select an output value from a list. To add further values, push <Ins> or the <space bar>. Push <Del> to remove values. Enter a header for the respective column in the report. You can make the width of the column wider than the header by inserting the appropriate number of blanks behind the header. Please note that the format defined by the column *Format* also affects the column width.

Substance Name of the substance or peak, respectively. The substance name is either

- the user entry after a rename command in manual evaluation
- a substance name from the component table identified by the peak position (retention time)
- consecutively numbered regions from the automatic peak search like R-002, the name may be defined in the setup.
- a vial range assigned from the fraction collector control 'M001-001'

Trace Name of the measured trace

Type Integration type (baseline type), see Manual evaluation.

Retention time (tr) Retention time of the peak.Retention time (tr)

The retention time of the peak is the time (or position) of the maximum value within the peak area. The maximum is calculated based on a 5 point smoothing. In case of a BKG region, the retention time is the middle of the region. If the middle is a half time interval, the left interval is taken.

Start (IS) Start of the integration region

Stop (IT) End point of the integration of the region. Stop is the time of the first interval not taken into account at the end of the region

Area (A) Integral area of the peak. The formula is:

$$A = \sum_{n=IS}^{IT-1} (I(n) - B(n)) * Dt$$

A: Area

IS: Start

IT: Stop, the integral does not include the last interval, this will be included to the next, contiguous region.

I(n): Interval values. These values can be obtained from the 'Values table'

during manual evaluation with the command 'CTRL-V'

B(n): Background values, see BKG for more information
Dt: Time interval, the duration of one time interval

%area	is the percentage of the peak area compared to the sum of all peak areas.
%total	Percentage of the peak area compared to the area above the baseline. This value is calculated for radioactivity traces only.
Concentration	Calibrated substance concentration. Available only after calibration.
Half-life width	Width of a peak at half of its height (FWHM).
Height	Height of the peak.
Net retention time (t')	Retention time of the peak minus the dead-time of the column.
Capacity factor (CF)	Cf = Tr / Dt Dt Dead time of column, Tr retention time of the peak
Limit of detection	Quotient of net retention time (tr) and dead-time of the column.
Number of plates	is 5.54 times the square of the quotient of retention time (tr) and half-life width (FWHM).
Symmetry	is the width of the left half of the peak on its 10% height divided by the width the right half of the peak on its 10% height.
Resolution	$RS = \frac{(tr1 - tr)}{(FWHM1 + FWHM)} * \sqrt{(5.54)}/4$ *tr* is the retention time of the peak, tr1 is the retention time of the following peak. FWHM and FWHM1 are the half-life widths of the peak and the following peak.
Selectivity	is the ratio of the capacity factors of the reference peak to the peak.
Internal standard	if several internal standards are used, this column indicates which internal standard was assigned to the substance.
Mean activity	Calculates the mean activity or intensity of the signal within the area
Counting error	Sqrt from brutto counts
Limit of detection	Minimum peak area which is secure detected at a given statistic (Sigma

value).

$$Li = f * n * \sqrt{(\sum_{i=IS}^{IT-1} B(i) * Dt)}$$

n Factor for sigma (1 sigma,3 sigma etc. to set in Setup / User options)

B(n) Background, see area for all other items

Relative retention T' / dead time of column. Set this time in the measurement method / Further parameters

Signal to noise ratio Theoretical value for the classical “noise”

$$Sig = 20 * \log(Area(net) / \sqrt{(area(brutto))})$$

Area(net): area as indicated above
Area(brutto): area without background subtracted

Footer lines

Footer lines contain summery information about the corresponding column.

Remainder (optional)

The remainder is the net content of a trace without the marked regions, thus it measures the activity not integrated. It is calculated for the columns 'Area' and '%Area'.

Total (only radio traces)

Total is area above the background (the area may be restricted to the the area between origin and front). If more than one BKG region is set, the baseline subtracted is a polygon between the points defined by each BKGn. Outside these points a constant value is subtracted defined by the outermost BKG. For TLC, two total values are calculated. Total means the whole measured area, while Total RF means the total area between 'START' and 'FRONT'.

BKG

Each BKG defines a point for the baseline to be subtracted. The position is the middle of the region. The height is the mean value of the regions intervals. The sum may be calculated like the Area but with all B(n) set to zero. The baseline for drop regions is defined by a polygon between the BKG regions. Outside the last BKG the baseline is constant.

$$BKG = \sum_{i=IS}^{IT-1} \left(\frac{(I(i) * dT)}{(T(bT) - T(bS))}\right)$$

I(i):Intensity at interval i, bS: start of background region, bT end of background region, T(i) time of interval i, dt: time of one interval

Peak search

For automatic peak search it might be necessary that you specify several values that describe the probable shape of the peak instead of using the automatic parameter detection

Trace	Shows the trace to set the parameters
Peak search method	Set the peak search method for the respective trace. The following methods are available: *No peak search* No peak search will be performed. Already set peaks will be retained. (This setting is also used to print a list of chromatograms with already set peaks using the automatic integration routine) *Automatic peak search* Automatic peak search is enabled. All peaks already set in the chromatogram are deleted before automatic peak search starts. *Auto + copy from component table* Automatic peak search is enabled. In addition, all peaks defined in the component table are copied into the chromatogram. This is useful to prove that substances are missing, for example in metabolite studies. *Copy from component table* No peak search is performed. Instead, the substances listed in the component table are copied into the chromatogram; the retention time and the time window define the start and end point of the integration region.
Control	The integration control influences the peak search and the integration. Here you can enter the values for peak width, baseline drift and minimum peak area for the entire chromatogram or for part of this chromatogram. See table below for details

Items to specifically influence the peak search

FWHM	Enter the FWHM (full width half maximum) of the peak. At this point, the width at half height need not be very accurate; an estimated value suffices in most cases. Enter “0” (zero) to force the program to determine the peak width automatically
Baseline drift	Enter here the approximate drift of the baseline per minute. GINA Star takes

	this to decide whether the baseline increases or a peak begins. Enter “0” (zero) to force the program to determine the baseline drift automatically.
Min. peak area	Minimum area indicates how high the contents of a peak has to be at least in order not to be rejected. You should increase this parameters if the peak search integrates pseudo peaks in the background noise of a chromatogram. The allowed range for peak reject extends from any small numbers (no peak found is rejected) up to any high numbers.
Auto-peak search	Disable or enable integration for a certain range of the chromatogram.
Peak direction	This setting allows the detection of negative peaks in certain ranges of the chromatogram. Possible values: “positive” and “negative”.
Force integration type	Force a certain integration type in certain ranges of the chromatogram. Possible values: “auto”, “baseline” and “drop baseline”. *Auto* setting defines the integration type according to the criteria defined in the program. *Baseline* forces integration regions of type “B” *Drop baseline* forces integration regions of type “D”.

Calibration Using Internal and External Standards

This chapter describes in detail the calibration with internal and external standards. You may use this feature to simply identify peaks or to calculate concentrations by help of external and internal standards.

Content calibration

Calibration Using Internal and External Standards....37
Overview....38
Calibration step by step....38
First step: separation....38
Second step: Enter standard concentrations....39
Third step: Do the calibration....39
Fourth (last) step: measurement of unknowns and do the recalibration....39
Important hint....40
Items of standardization....40

List of components....40
Calibration curve....41
Calculation rules....41
Calibration via Internal and External Standards....42
Determining the calibration curve....43
Calculation of concentration of a substance in unknown samples....43
Internal and external standards....43
Calculation of calibration curve....43
Calculation of the concentration of an unknown sample....44
Entries for calibration....44
External and calibrated internal standards....44
Calculation of the calibration curve for the internal standard....44
Calculation of the concentration of an unknown sample....44
Operation....45
Weighted Calibration Points....45
Trouble shooting for standardization....46

Overview

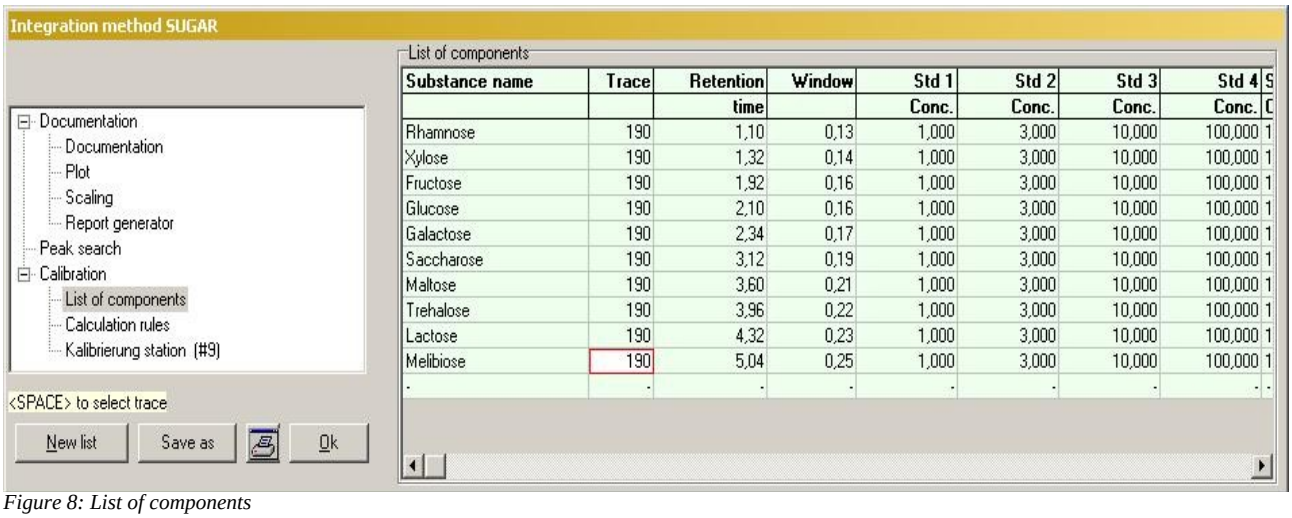
External standardization employes the result of a measurement with known concentrations to determine the sensitivity of the instrument against a substance to calculate concentrations from unknown samples in subsequent measurements. After establishing the method, the only task of the user is to tell GINA Star whether the next sample in the sample table is a known concentration (Standard) or an unknown concentration.

Calibration step by step

As a part of GINA Star's installation the method 'sugar' appears with some standard measurements and one unknown. This allows to follow the steps without doing real measurements.

First step: separation

The first step of a calibration is a chromatographic separation of some components in a normal chromatogram. Let peak search find the peaks and rename them to reflect the name of the corresponding component. After this from the chromatogram view you open the integration method, select 'List of components' and push the button 'New list' on the left hand part of the screen. By this, GINA Star copies information from the chromatogram to the components table. These are the positions and names of all peaks:


Integration method SUGAR
List of components
Substance name | Trace | Retention time | Window | Std 1 Conc. | Std 2 Conc. | Std 3 Conc. | Std 4 Conc.
Rhamnose 190 1,10 0,13 1,000 3,000 10,000 100,000
Xylose 190 1,32 0,14 1,000 3,000 10,000 100,000
Fructose 190 1,92 0,16 1,000 3,000 10,000 100,000
Glucose 190 2,10 0,16 1,000 3,000 10,000 100,000
Galactose 190 2,34 0,17 1,000 3,000 10,000 100,000
Saccharose 190 3,12 0,19 1,000 3,000 10,000 100,000
Maltose 190 3,60 0,21 1,000 3,000 10,000 100,000
Trehalose 190 3,96 0,22 1,000 3,000 10,000 100,000
Lactose 190 4,32 0,23 1,000 3,000 10,000 100,000
Melibiose 190 5,04 0,25 1,000 3,000 10,000 100,000
Documentation
Documentation
Plot
Scaling
Report generator
Peak search
Calibration
List of components
Calculation rules
Kalibrierung station (#9)
<SPACE> to select trace
New list
Save as
Ok
Figure 8: List of components

Second step: Enter standard concentrations

Standard concentrations have to be entered to the 'List of components'. Standard solutions have numbers, each should have a different concentration. Normally standard solutions derive from a first mixture by diluting. Thus, they have a fixed relationship.

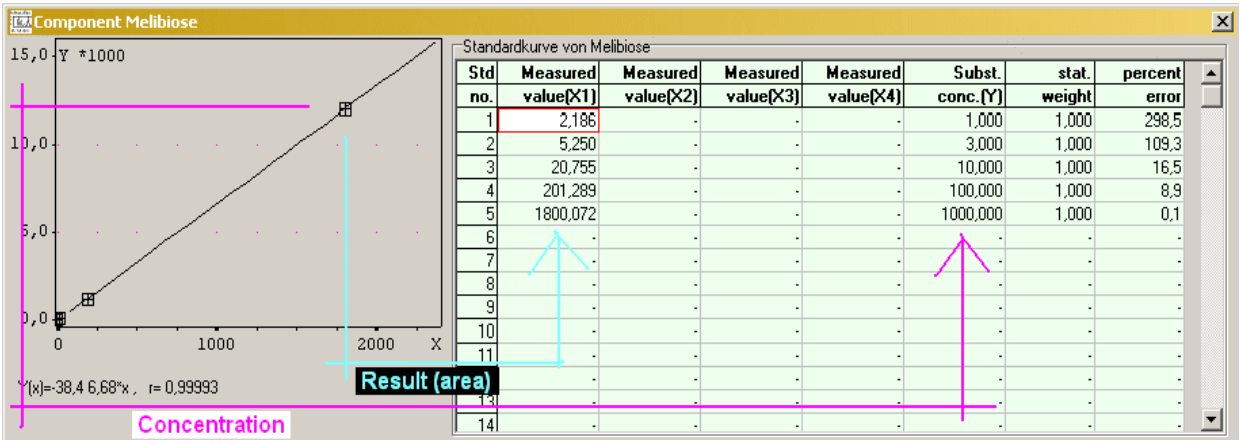


Figure 9: External standard component with curve

Third step: Do the calibration

The very first time, measurement of standards are used to establish the sample table. Thus they have not already been used to for calibration, this has to be done manually this time. To do this, again open the measurements, from the 'Edit' or from the context menu, select 'Information'. Now select the 'Calibration' tab.

Fourth (last) step: measurement of unknowns and do the recalibration

Unknown samples may now be processed without taking care about anything. The column 'Conecentration' contains what GINA Star looks up from the above curve. To start a new

calibration, select the appropriate standard number in the sample table. The first standard in a series of standards clears the whole calibration, no part of the old calibration remains valid over the start of a new one. All standards have to be measured within one block with no intermittent samples. If you don't want this, don't mark standards in the sample table and enter them to the curve like in the previous, third step.

Important hint

Once a report is printed of an unknown run, the result table of the actual calibration is copied to the run itself. If you want changes on the actual calibration to be reflected in that run, open the Information, TAB calibration and press button Return to calibration of station.

Items of standardization

List of components

Substance name	Enter the name of the component. This name links the regions with the standard values and curve, but may also simply be used for peak identification.
Internal standard	Enter “I” when the substance serves as internal standard. If more than one internal standard is allowed (see chapter on page), they are numbered automatically. Each individual component refers to an internal standard. Enter the number of the internal standard on which calculation of the respective substance should be based. If only one internal standard is foreseen, this assignment is not required.
Trace	Enter the trace to which the substance should be assigned.
Retention time	Enter the retention time of the substance. If you enter “N” instead of the retention time, the identification will be based on the peak name.
Window	If you want to define an individual window for the retention time instead of the absolute and relative windows, which can be set in , enter it here. Under normal circumstances the width of the window is calculated based on the information for “absolute window” and “relative window” from the menu . However, if this value has been overwritten once, you have to enter a zero to restore it again.
Concentration	Enter the concentration of the calibration substance in the respective standard solution. In you have defined one unit (for example ug/l). Use this unit. The number of the standard has to agree with the respective

information in the . Push <F2> to process the calibration curve.

New list

This is a button below the left hand menu and allows you to automatically generate a table of substances of standard solutions from the evaluation you came from. A standard solution must have been measured already. After invoking this menu item, enter the name of this measurement. The list of sample shares is generated from the integration regions set. You only have to enter the amounts or concentrations, respectively, of the substances in this list.

Calibration curve

This window (see Figure 2) contains the calibration curve along with the curve parameters and the respective measured values.

Measured value

Measured value (area or height) from standard measurement. Under normal circumstances, this value should not be changed manually.

Substance concentration

Concentration of the component of the respective standard solution. Same as the value in the component table.

Injection volume

Injects sample volume as indicated in the measurement sample table.

Statistical weight

You have the option to assign a statistical weight to the individual calibration points. Push the <F2> button to assign statistical weights automatically. Select between constant distribution, 1/x and 1/x2, where x is the concentration.

Percentage error

Distance between calculated curve and measrured value

Presentation of the calibration curve can be spread gradually by pushing <F5>, or compressed again by pushing <F6>; push <F3> to reset it to its original scaling.

Calculation rules

Calculation rules affect the algorithm to calculate the concentration curves.

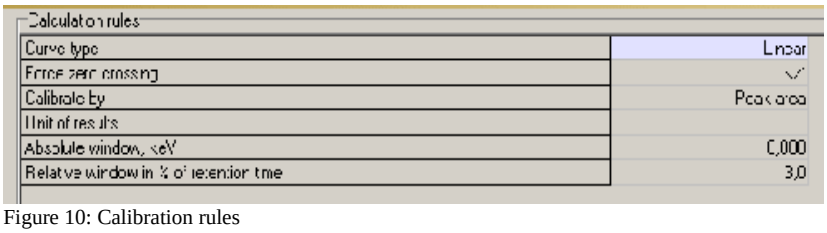


Figure 10: Calibration rules

Curve type	Defines whether the curve is linear or not. Normally it should be linear.
Force zero crossing	Specify if the standard curve should pass through the zero point or if it should be allowed to cut the axes at another point.
Calibrate by peak area or peak height	Select if calibration calculations are to be performed based on peak area or peak height.
Unit of results	The unit for calibration serves for documentation and as an ensurence while input of values. The unit is irrelevant for calculation, the results have always the same unit, just like the entered standard concentrations.
Absolute window	The retention time from the component table is used to identify a substance. However, since the retention time may vary slightly, a retention time range or retention time window has to be defined for identification. Enter here the permitted deviation in positive and negative direction in absolute time units
Relative window	The retention time from the component table is used to identify a substance. However, since the retention time may vary slightly, a retention time range or retention time window has to be defined for identification. This value is added to the absolute window. ith increasing retention time, this variation increases as well. Therefore, we have provided the option to make the size of the window dependent on the retention time. Enter the size of the window in percent of the retention time. Please note that the absolute window will be added to this window

Calibration via Internal and External Standards

The program offers different ways of calibrating results via internal and external standards. This chapter will describe these procedures, explains their use and shows how results are derived from raw data.

The following definition will be referred to in the following chapters. For each sample (standard or unknown sample) the sample amount is calculated in arbitrary units:

$$Sample\ amount = \frac{InjectionVolume * Weight}{Dilution}$$

Measurement result refers to the area of a substance peak or to its height, depending on whether calibration via *Peak areas* or *Peak heights* has been set in the integration method in the menu *Calculations rules*.

The GINA program allows the use of up to four internal standards. This fact will not be pointed out any more explicitly in the following chapters. Which of the four internal standards has been used for calibration of the respective substance is defined in the component table. See chapter on page .

Determining the calibration curve

A series of standards with known substance concentrations will be measured for calibration with external standards.

To define the calibration curve, the concentration of the component from the component table is multiplied by the sample amount and plotted against the measured value. A polynomial of regression *f (measurement value)* is drawn through the measured points according to the least mean square error method taking into account statistical weights of the individual measurement points. This polynomial is maximum one of the third degree.

If the calibration curve should pass through the zero point, the zero point is automatically added as additional measurement point to the measured points. This additional point is given a statistical weight of 1000 above the highest statistical weight of the calibration.

Calculation of concentration of a substance in unknown samples

If you are working only with internal and not with external standards, the substance concentration of the unknown component is calculated as follows:

$$\text{Concentration} = \text{measured value} * \frac{\text{Conc}_{\text{IntStd}}}{\text{measured value}_{\text{IntStd}}}$$

Enter the internal standard substance with name, retention time and retention time window in the component table. Enter “I” in the column *IntStd*. Thus, this substance is defined as internal standard substance. In this row, the columns *Std1 Conc* to *Std16 Conc* will be left blank, i.e. they will contain only zeros.

If you plan to use more than one internal standard, you have to enter each internal standard substance in this manner in the component table. The external standards are numbered automatically. All substances that are to be included in the calibration, also have to be entered in the component table. In the column *IntStd* you enter the digits “1” to “4” to define to which internal standard they should refer to. The retention time and the retention time window are also entered. Only columns *Std1 Conc* to *Std16 Conc* have to contain zeros!

In the sample table you have to enter the concentration of the respective internal standard in columns *Int* or *Int1* to *Int4*.

Internal and external standards

This method combines the calculations of the external standard method discussed above and pure internal standard method. Calculation of the calibration function is similar to the external standard method:

Calculation of calibration curve

For calculation of the calibration curve the concentration of the component from the component table is multiplied with the sample amount, divided by the concentration of the internal standard and

plotted against the quotient of measured value of the component and measured value of the internal standard. A polynomial of regression *f (measured value/measured value*$_{IntStd}$*)* is drawn through the measured points according to the least mean square error method taking into account statistical weights of the individual measurement points. This polynomial is maximum one of the third degree.

Calculation of the concentration of an unknown sample

The concentration is calculated from the measured value (area or height of substance peak) of a component in an unknown sample:

$$Conc = \frac{Conc_{IntStd} * f(\text{measured value}/\text{measured value}_{IntStd})}{\text{sample amount}}$$

Entries for calibration

Enter the internal and external standards in the component table as described above: in the column *IntStd* you define the internal standards and the correlation of substances and various internal standards (if more than one internal standard is used). The columns *Std1 Conc* to *Std16 Conc* of the internal standards have to contain zeros. For the external standard components, these columns contain the respective substance concentration of the respective concentration level.

In column *Type* of the sample table, define the external standard samples with *Std1* to *Std16*. Enter the concentration of the internal standard substances in the columns *Int* or *Int1* to *Int4*.

External and calibrated internal standards

Besides calibration of the substances to be measured, *GINA - STAR* also allows calibration of the internal standard substances using known external standards. This method is to be used when the internal standard is added to the samples in different concentrations and it is to be expected that the measured values of the internal standard substances are not proportional to their concentrations. In many cases, this can be assumed with the same right as it is assumed for the unknown substance concentrations to be measured and one applies to them a calibration via external standards.

Calculation of the calibration curve for the internal standard

If you have created a calibration curve for the internal standards, the calibration curves for the internal standard substances as well as for the substances whose concentration it to be determined is calculated according to the external standard method described above.

Calculation of the concentration of an unknown sample

The calculation of the substance concentration of an unknown sample is done according to the formula:

$$Conc = \frac{Conc_{IntStd} * f(\text{measured value})}{f_{IntStd}(\text{measured value}_{IntStd})}$$

Operation

As described in the previous chapters, the internal standards, the references of the other substances to the respective internal standards and the concentrations of the standard substances in the respective standard samples are defined in the component table. The difference is, however, that the columns *Stdn Conc* have to be filled in for the internal standard substances. Enter the concentrations of the internal standard substances in the respective standard solutions.

These concentrations will be copied automatically into the sample table in the lines of the standard measurements. The columns *Int* or *Int1* to *Int4* cannot be changed in those rows including a standard as sample type. However, in the rows defining a measurement of *type sample*, you have to enter the concentration of the internal standard substance or internal standard substances in the columns *Int* or *Int1* to *Int4*.

Weighted Calibration Points

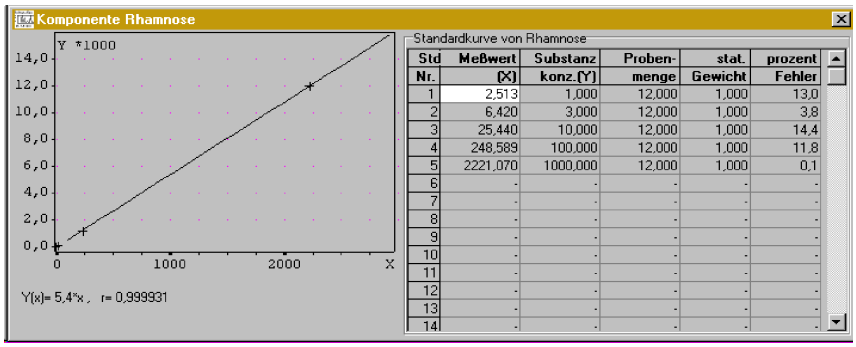
In the menu *Integration method/Edit calibration curve* you find the column *Statistical weight* which allows you to assign a weight to each calibration point for calculation of the calibration curve.

The purpose of this feature shall be explained by the following example. The figure below shows the calibration curve for the substance Rhamnose:

The optical impression of the curve as well as the correlation coefficient of 0.99888 appear to indicate that the calibration is correct. However, if you look at the column *Percent error* you will find an error of 271.6 % for the concentration range of 1.000.

How can this phenomenon be explained? The straight line, which passes through the calibration points, is the result of a *linear regression*. This mathematical procedure fits a straight line in a group of data points, minimizing the sum of the (absolute) error squares. By using the absolute error we get an overvaluation of the high measured values. The consequence is a larger (relative, i.e. percentage) error for smaller measured values, i.e. for lower concentrations.

We can view this effect better when we spread the curve by pushing the button <F5>. If you push <F5> five times, the presentation looks as follows:



Std Nr.	Meßwert (X)	Substanz konz.(Y)	Proben-menge	stat. Gewicht	prozent Fehler
1	2,513	1,000	12,000	1,000	13,0
2	6,420	3,000	12,000	1,000	3,8
3	25,440	10,000	12,000	1,000	14,4
4	248,589	100,000	12,000	1,000	11,8
5	2221,070	1000,000	12,000	1,000	0,1
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-

Now only the first two calibration points are displayed. One can see how far they lie next to the calibration curve.

Move the cursor to the column *Statistical weight*, push <F2> and enter statistical weights for each calibration point. However, you can also perform automatic weighing. In the column *Statistical weight*, push the <F2> button and select one of the offered weighting functions. In our example, we select 1/x2. This means that the weight is calculated by the reciprocal of the square of the substance concentration.

The result is displayed immediately:

Now the curve passes correctly through the calibration points, the calculated percentage error for standard 1 (concentration 1.000) has dropped from 271.6 % to 1.1 %! For compensation, the error of the third standard has increased from 0.2 % to 3.5 %.

Conclusion: Due to the commonly used mathematical method of linear regression for calculation of a calibration curve based on calibration points (external standards), there is an unequal distribution of relative errors in the low concentration range and thus a high relative error.

A more even distribution of relative errors over the whole concentration range can be obtained by applying statistical weights to the calibration points.

<F5> spreads the curve; pushing <F5> repeatedly increases the spreading. <F6> gradually undoes the spreading, whereas <F3> cancels the spreading

Trouble shooting for standardization

If you do not get the desired results, please check the following items prior. to calling the raytest service.

Options enabled / menu items don't appear	Are the needed items displayed in the dialog? If not, please check Setup / User options / Dialogs whether external standard is enabled and the needed number of internal standards.
No entry in result	Be sure to have the correct standard number in the sample table. After measurement you may check and correct by the command Edit/Information/ Calibration.

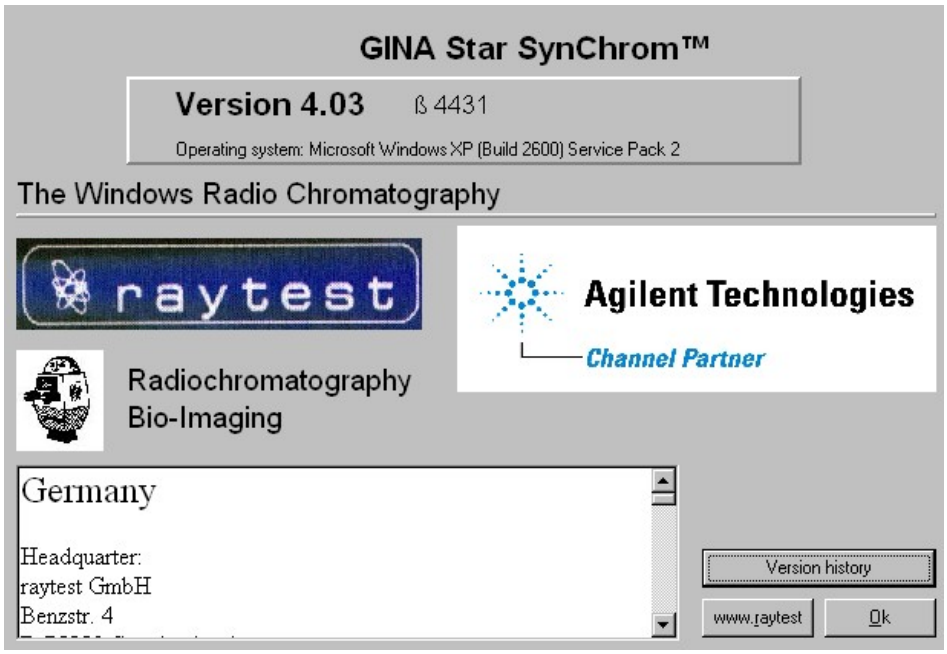
table	
No result for 'unknown' or normal samples	- Did you add the 'Concentration' column in the report generator? - Do you have peaks with the name of defined components? - Did you calibrate before obtaining results? Otherwise enter Information / Calibration and 'Return to calibration of the station'
Units don't appear	Units should be entered to the page 'calculation rules' within the evaluation method. They are for documentation only because GINA Star calculates the results in the same unit as the standard concentrations are given.

Support and Version Management

Examine the program version

Information about the software version is provided on the version information dialog. You gain this by the menu

Help / Info



Picture 11: Version information

The first line contains the name of the software, the second line contains the exact version number:

Releases	Releases have a release certificate at the end of a test phase. Releases are indicated just by the version number like **Version 4.xx**
Bug fix	Bug fixes are guaranteed to be derived from the corresponding release only by fixing bugs. These bugs are listed in the version history. **Version 4.xx** release 2
Development version	Development versions contain new features and you should only use development versions, if your work depends on these new features. Version with new features are not guaranteed to work correctly. The indication is **Version 4.xx** dev. bbbb. bbbb is the build number of the version and is incremented for every build.
ß - releases	ß - releases are the end of a development of new features. The software is not yet fully tested. If you use a ß, hints about problems are especially welcome. **Version 4.xx** ß bbbb. bbbb is the build number from the same series of numbers like development build numbers.

Support

Menu 'File / Problem report'

To give support, sometimes setup information or run data are necessary to analyze a problem. To

make the easy, GINA Star does the installation of 7-zip all necessary data to one zip file. If you enter this menu without having a run opened, only the setup is copied, otherwise the actual run data are copied as well.

The compressed ZIP automatically opens in the explorer and you may now e-mail this by help of the context menu or you may copy it to the network or to a memory stick to send it from elsewhere.

Please give a hint in the text field about your problem even the report is announced by a telephone call.

Important notice:

Support normally needs a lot of information from the customer side. For all information provided to the software development is guaranteed: this information is always regarded as confidential and used only to solve the problem described by the customer. Because

Security and Control

Security means internal proof mechanisms to ensure data integrity and security against manipulation. These features are designed to fulfill the regulations of the FDA regulations CFR 21 Part 11.

GINA Star data Security and Control

Security and Control....50
Access control....51
Dialogs for users and rules....51
User list....51
User account....52
Certificates for user defined signatures....52
Rights within the rules....52
User classes....53
Signatures for run and evaluation data....54
How to approve and unsign measurements....54
Theory of data security for GINA Star 4....54
Measurement data....54
Imported Data....55
User signing of RAW data....55
Evaluation data....55
Certified data....55
Signatures by Certificates in the Windows Certificate Store....56
Hint for the software validation:....56
Audit Trails....56
Audit trail viewer....56
Measurement audit trail....56

Measurement method audit trail....57
Evaluation audit trail....57
Global trails....57
User access audit trail....57
Instrument control trail....57
Critical events trail (Sytem trail)....57

Access control

Access control regulates the access to various functions. To accomplish this, users are organized in classes. Rights are defined within these classes.

- From the menu call Setup / Edit setup / Program / Access control

Dialogs for users and rules

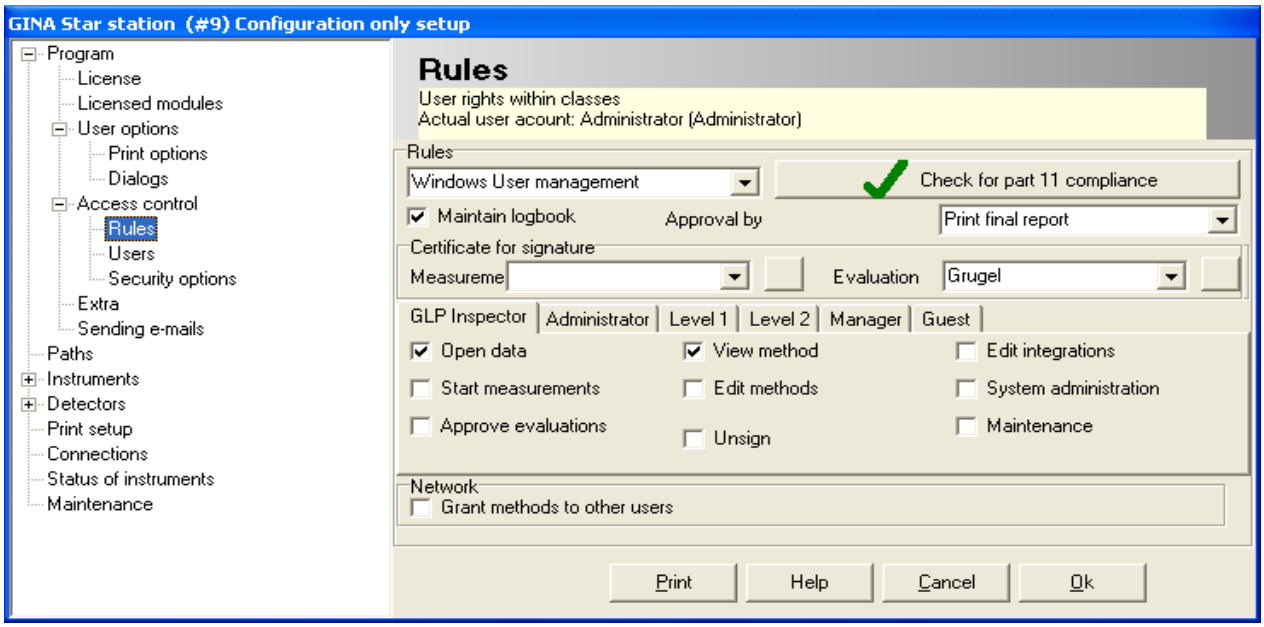


Figure 12: Access control

Each users belonging to a class is indicated in the user tables column 'Allowed access'. The class is selected from a drop down list and belongs to rights within a tab on the 'Rules' page. Here the rights are granted by checking the corresponding check boxes.

User list

The user list contains information about access rights to GINA Star for each individual user. User names are case insensitive. The actual user is selected from this list

User account

All user account setup properties are valid for all stations using the same setup root directory except the user certificate for signing the evaluation.

The 'Rules' area starts with a check box to select the type of user account. No access control disables the account control. Everything is allowed to any user. Trailing access is disabled. The recommended access control is 'Windows User Management'. This means, GINA Star uses the account formerly used to login to Windows 2000 or Windows XP. Thus password management and checking is done by the OS.

GINA User management means, passwords and account are maintained by GINA Star independent of the OS. Thus, every time GINA Star is started, the user is asked for a user name and password. The GINA Star user management is not checked for compliance.

Certificates for user defined signatures

See also: Signatures

If the 'Windows User Management' access type is selected, GINA Star offers two selection drop downs from the windows certificate store. The first belongs to measurements. GINA Star guarantees the data integrity for measurements acquired with version 4.01 and higher and data files imported from actual versions of other raytest products. Data import from older versions of GINA Star as well as imports from foreign software cannot be checked, GINA Star cannot guarantee for untouched original data. So far these data cannot be approved and signed at the end of the evaluation. As a substitute for this, the user may sign the measurement by his name. This means he guarantees the data integrity instead of GINA Star. In this case GINA Star allows again to approve and sign the evaluation.

The second certificate adds up the signing of evaluations. Signing the evaluation always employs a certificate build in to GINA Star. In addition GINA Star takes a user certificate selected here to add a second signature to the evaluation. The improvement of this is, that the user is able to proof even outside the company, that the data belong to a certificate tree of the companies CA.

Rights within the rules

Open data	View the measurement results or evaluated data
View method	This right allows to view measurement methods.
Change evaluation	This right includes the right to change evaluation methods as well as the measurement file directly. Change evaluation includes the right to delete non-controlled runs. At the end, this right allows to 'complete evaluation'. This right automatically implies 'Open data'.
Start measurements	Allow to start single measurements or to start the execution of a sample table. This right automatically implies 'View method'.

Edit methods	This right grants the users the right to edit and change measurement methods. This right automatically implies 'View method'.
System administration	This allows to manage exact these pages of information, the user list with the belonging to classes, and the rights within these classes.
Approve evaluation	This is the main function of the documentation, signing the document. In the case you employ a smart card with a fully qualified certificate, this is equal to a hand written sign to that document. This right automatically implies 'Open data'.
Unsign evaluation	To re-edit a evaluation already signed, it is necessary to unsign it. This depends on this special right. In mixed organizations using paperless and printed documents, the printouts have to be marked at this point. If users with allowance to edit evaluation try to edit a 'completed evaluation', it depends on this right to do that. Unsign is done from context menu 'Information / Evaluation'. Here, buttons are available for sign or unsign. This right automatically implies 'Open data'.
Maintenance	Allow the maintenance of the instruments, i.e. Change the UV-lamp of the UV-monitor. Of course GINA Star can only observe the access to the use counters of these items.

User classes

In opposite to groups, classed contain users only once, each user belongs exactly to one class. This may be less flexible as groups, nevertheless it is more transparent. Access control consists of the user table and the rules tabs.

The 'Levels' have some freedom to adapt special rules in the customers lab. By default, Level 1 is a method developer and has the right to edit measurement methods, start measurements and do all evaluations.

User classes have a name and default rights already assigned to have a read to use system for standard situations

GLP - Inspector	The GLP – Inspector needs to view all data and should not have the right to take any influence.
Administrator	The administrator has to manage the user accounts and the user rights, but normally should not process or edit measurements. He may as well have the right to maintain the system.

Level 1	The 'Levels' have some freedom to adapt special rules in the customers lab. By default, Level 1 contains users, which are allowed to do evaluations. This includes edit of evaluation methods.
Level 2	Allow to start single measurements or to start the execution of a sample table.
Manager	The manager is by default the only one to unsign measurements. He is also allowed to influence evaluations.
Guest	The guest may only view measurements.

Signatures for run and evaluation data

How to approve and unsign measurements

At the end of the evaluation, data may be signed (approval). Signing data works in two steps. Users having the right to do the evaluation apply a “Mark evaluation completed” to indicate, evaluation is ready. Users having the right to apply the signature (approval) by the same buttons. A user may have both rights, in this case both steps are done at once.

The rightmost icon in the icon bar offers the approval.

From evaluation call context menu 'Information' view on the tab 'Evaluation', an approval button is offered

- From the catalog select all desired runs and from the context menu select 'Approve evaluations'.
- To unsign a measurement, call Edit / Information, select tab 'Evaluation' and press the 'Unsign' button. This is available to revoke a normal approval as well as to revoke the status 'Evaluation completed'.

As the result of the approval, the icon of the MDI title bar gets the green “ok” symbol (this is thin, if the evaluation is marked completed). This symbol will indicate the state of approval in the catalog as well.

Theory of data security for GINA Star 4

Measurement data

Instead of a checksum, a signature from a certified 1024 bit RSA key pair is used to maintain data security. Raytest guarantees, that RAW data are not influenced as long as the corresponding

signature is verified by GINA Star.

Contributing to the hash and therefore protected are: Run name, Method name, Start time, Raw data including trace names, unit names, Instrument name and information about the connected instruments, their status, serial number and the build number of the GINA Star release..

Imported Data.

Data imported from other instruments (i.e. MiniGITA) need to have a signature and GINA Star should be enabled to verify this signature. In this case GINA Star will sign the imported data after conversion. Imported data not containing a signature may be signed after import by help of a user certificate within the Windows certificate store.

User signing of RAW data

GINA 4 Star will allow to approve data only, if the integrity of the RAW data is proofed. This is guaranteed for all runs driven by GINA Star 4. Imported data even from 2.18 and 3.00 or earlier do not have this guarantee. Thy are indicated by the yellow '-' in the symbol (catalog and title bar) Runs with affected RAW data have a red symbol .

To allow approval of these data, the user may sign RAW data of runs by help of a user certificate in the windows certificate store. This certificate has to be supplied from the customers side (test certificates are available from raytest).

Evaluation data

A signature can only be applied, if the measurement data have a signature.

For the evaluation data the signature is not generated automatically but by an indication of the user that the evaluation process is finished. Due to an option this happens while printing the 'Final report' or explicitly by the 'Sign' button on the Signatures tab. If a smart card CSP is selected, the smart card must be present during signing data.

Any operation changing evaluation data and thus destroying the signature need a confirmation. The signatures becomes invalid, but will not be changed. Thus revoking the changes by any way will re enable the signature.

Please note this warning: It is possible to use certificates, which private key usage requires confirmation (PIN, ok- button). Some security environments block the thread system for this wait time. This situation cannot be predicted by GINA Star and will damage a run underway.

Certified data

A data along with signature will be displayed at their normal places in the report and the 'Information' register. A data and time will only be displayed here, if a time stamp server was employed to verify the time.

If future releases of GINA Star will take more or other information to calculate the MD5 digest, GINA Star will calculate digests from old versions version dependent to keep the signatures valid.

For the measurement following data contribute to the digest:

Injection time, run name, instrument name, study name, build number of GINA Star running the measurement,

For the evaluation following data contribute to the digest:

Injection time, run name, instrument name, study name, build number of GINA Star running the evaluation,

Signatures by Certificates in the Windows Certificate Store

In addition to the key pair hidden in GINA Star, GINA will employ a user's certificate of the Windows certificate store to sign data. Every time GINA Star signs evaluation data, it will look for a certificate selected in GINA Star's setup. If such a certificate containing a private and a public key pair will exist, GINA Star will use it to generate a second signature by help of the ESP (encryption service provider). This functionality is available only within Windows 2000 professional or Windows XP professional.

Hint for the software validation:

Signing must not work without private key. To test this, it is not sufficient to delete the certificate from the store and import the variant without private key. This is a known property, bug or feature of the windows cert store, the private key is retained while deleting the certificate. The only way to delete the key is to export with the option 'Delete private key from store'.

Audit Trails

Audit trails log actions to create documents containing information like 'who did what at which time'. Some log files belong to a measurement or a measurement method. All others are system level, they may be opened and viewed from the main menu.

Audit trail viewer

The audit trail viewer grants a view to the system log files. The viewer allows for file update during view. To use other viewers, please export the data by the copy button and paste it to that editor.

Measurement audit trail

The measurement audit trail contains information about controlling chromatographic parameters. The content is defined by the method and by possible manual changes during the run. Thus the information is redundant and not printed in the report by default.

Measurement method audit trail

This trail covers every change on any item of the measurement method including creating and copying. Thus it may be helpful instrument during method development.

Evaluation audit trail

The evaluation audit trail lists all steps leading to the results. It is part of the measurement file and will not be printed in the report by default.

Global trails

Global trails contain events not directly associated to a run. These trails are stored in the stations directory.

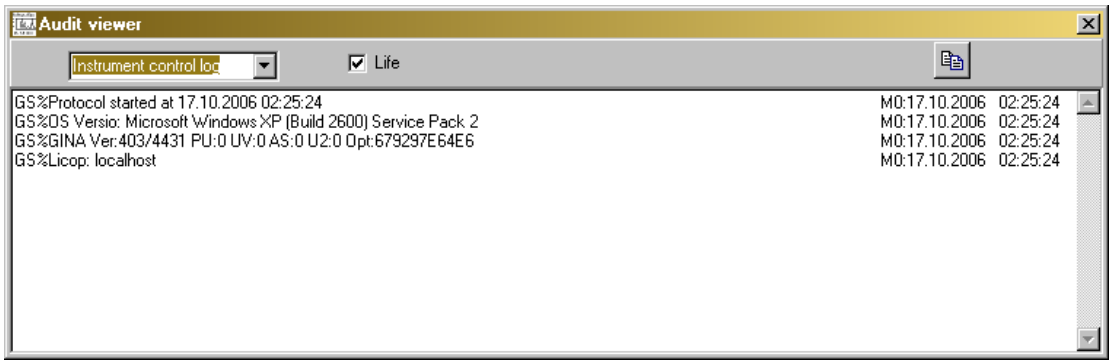


Figure 13: Audit trail viewer

User access audit trail

The user access trail logs every controlled access and login / logout processes nevertheless action is allowed or refused.

Instrument control trail

Instrument control trail contains information about the communication with the connected instruments like pump, UV-monitor etc. Every command and every response from the instrument is reported here. Periodic status messages may be filtered to appear only once per 10 occurrences. The length of this trail is limited to somewhat more than 1 MB, 20 of these trails are retained, normally enough for 10 to 20 days. To clarify problems, it is helpful to do this within this period.

Critical events trail (Sytem trail)

Beside some framing information ('GINA Star Version xxxx started..') this trail contains information about exceptions in the software. This information is especially needed, if problem diagnosis is necessary.

Setup GINA Star

The GINA Star setup contains the installation, license, user preferences and connection information for the various components.

Contents of GINA Star setup

Setup GINA Star....58
- Program Installation....58
  - Program Start....59
  - Update an existing GINA-DOS or NINA-DOS installation....59
  - Compatibility....59
  - Warranty notice....60
  - Forms of delivery....60
  - Requirements to the system....60
  - Windows user accounts....60
    - Start parameters....61
  - Organization of Data....61
  - Station (or instrument) name....62
- Setup GINA Star....62
  - License....62
    - Serial number....63
    - License number....63
    - License addendum....63
    - License....64
  - Paths....64
  - Print options....65
  - Dialogs....65
  - Autosampler....67
  - Connect autosamplers....67
  - Connections....67
  - Trouble shooting....68
- Using fraction collector....68
  - Setup and connect the fraction collector....69
  - Output signals....69
- Maintenance status....70
- GINA - STAR Data Acquisition Box....71
    - Connecting Detectors....72

Program Installation

The program CD includes an automatic installation routine (’Setup’). It creates or updates the complete installation “GINA - STAR” on your hard disk. It includes some demonstration files that help you to get a

first impression of the program. Program versions prior to 4.00 are not updated. Instead a second installation is created. See Compatibility for compatibility with Versions 2.xx and 3.00.

Program Start

After installation, the GINA - STAR icon is displayed on your computers desktop. Click this icon to start GINA - STAR. GINA Star will appear with the menu and an empty MDI frame.

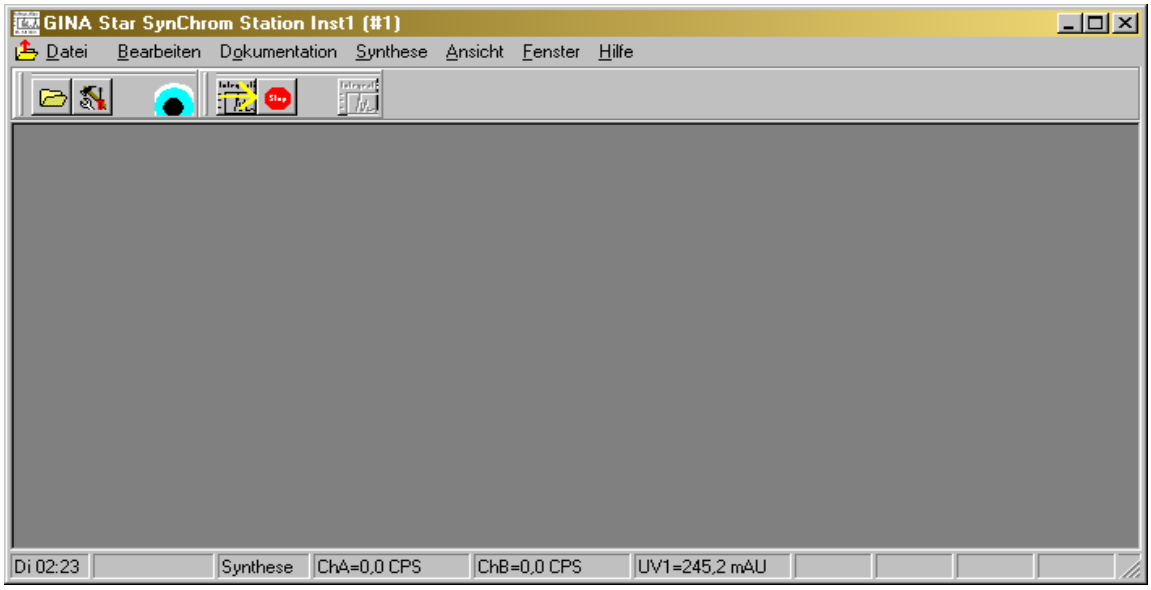


Figure 1 GINA main menu

Now GINA – Star may be used by either the menu functions by help of the mouse, the standard key commands or by accelerator keys for a lot of commands.

Update an existing GINA-DOS or NINA-DOS installation

Copy the dos file structure ('c:\gina') to c:\gina_nt. GINA Star will use the setup and all other files except the license. Ask for a new license and provide the serial number of your GINA Box.

Compatibility

GINA Star 4.0 reads data files from all previous versions of GINA, Ramona. For TLC data, a special version is available. Because GINA Star 4.0 uses new data formats for most items, there is no backward compatibility to previous versions of GINA Star. This means, data created or edited by GINA Star 4.0 will

not be readable by GINA 2.18 and GINA 3.00.

The converter for GINA 2.18 methods or 3.00 methods to GINA 4.0 methods is complex. For this reason, GINA Star 4.0 does not offer these methods directly to start a measurement (single measurement or sample table). Instead you have to open the method and check whether it is correct after conversion. After saving, this method is available for new runs.

Warranty notice

The installation of GINA - STAR can be carried out by the supplier or by the customer. Installation by the customer does not limit the warranty for the complete system and its performance. Nevertheless, assistance during the installation phase may be charged by raytest, if installation is not purchased.

Forms of delivery

Installation of the GINA – Star Chromato-Graphic systems is carried out via the setup program *'Setup'*. This is available on a CD as well as from the Internet under www.raytest.de under download / GINA Star. In some cases this will come along with a configuration for your system. GINA Star will automatically use this, if the CD remains in the drive until the first start of GINA Star. You may later use the menu 'File / Setup / Load setup' to read the setup configuration from the CD '\station.customer'.

Requirements to the system

- The program is running under Windows XP professional and Windows 2000 professional. (home versions as well as Windows 95/98 and Windows NT do not support all features of GINA Star nor does raytest test on these OSs).
- The available memory should be the amount advised for the operating system
- We recommend a monitor resolution of at least 1024 x 678 pixels.
- The OS must not have support multiple processors enabled. GINA Star recognizes this situation and will not run. See hyperthreading memorandum for more information.

Windows user accounts

Some setup entities of GINA - STAR depend on the user account to Windows. These are i.g. the selected printer, saved window properties (size and position) and the location GINA - STAR stores all other setup properties including your license. If you start windows with a new user account, GINA - STAR while starting the first time will ask you for the directory for saving the setup. This is 'C:\GINA_NT' by default. If you select the same directory for every account, GINA - STAR uses the same setup for every user account. Please ensure, that user accounts do not have different drive mappings.

Start parameters

GINA - STAR accepts one or more data set names as start parameter. This makes it possible to paste a data set via drag and drop onto the GINA - STAR icon on the desktop. If a GINA Star is already running, this file is passed to the running GINA Star.

/c	Invokes the configuration only mode of GINA Star. In this mode GINA Star does not try to connect to any instrument
/ee	With the following files execute an evaluation.
/pr	Print the following files
/on	Open the following files
#n	Station number identifies a setup for one station.

Operation of Several Systems on one PC or one Network drive

You can operate several systems on one computer or one network drive at the same time. To do this on one computer, copy the start icon on your desktop. Rename the second icon, e.g. 'GINA_NT System 2' using the context menu 'properties'.

Add the station number i.g. #10 to the destination
"C:\Program Files\Raytest\GINA_NT\GINA_NT.exe" #10

GINA Star started with this icon now has it's own setup. Please use Setup / Extra / instrument name, to enter a specific name. This will appear in the title bar, the task bar and in a future list of stations.

Organization of Data

A large number of data sets has to be organized such that data can be easily located, opened and archived easily. GINA - STAR offers two organization methods which are selected in Setup / Paths.

Setup	c:\gina_nt\station.loc\setup.gina, c:\gina_nt\station.loc\instrument.nam, c:\gina_nt\station.glb\lRules.rDat
Methods	i.g. c:\gina_nt\method\sample The method describes the conditions under which the measurement is be performed. At the same time, the method name is used to create a directory in which the associated measurement data are stored. If selected in the paths menu, there is again a subfolder with name of the station, here are the methods.
Study	i.g. c:\gina_nt\study\method\ sample

	If you select this option, you enter a study name for each sample in the sample table.
Instrument	i.g. c:\gina_nt\study\instrument\method\ sample The instrument name is a fixed name for each station. From version 4, this is identical to the station name.

Station (or instrument) name

GINA Star uses one name for the station. This name is reflected in GINA Star's title bar, the task bar, the title of the instrument's control screen and some other places to identify, which system is controlled (at least this is useful if you drive more than one station on one computer). If selected, this name becomes part of the method path and run data path

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Setup GINA Star

GINA Star uses an registry entry HEKY_CURRENT_USER/software/raytest/gina_nt/GINALOC to address the GINA Star setup directory <GINASetup>. This is c:\GINA_NT by default. Within this directory, a file setup.glb\lRules.rDat contains common setup data for all stations. These are security rules, user trusties, access rights and certificates. The file <GINASetup>\station.loc\setup.gina contains all information about one station, connected instruments, user options etc.

License

Licensing allows to control the use of the software and software options. Licenses are granted by raytest GmbH, Germany.

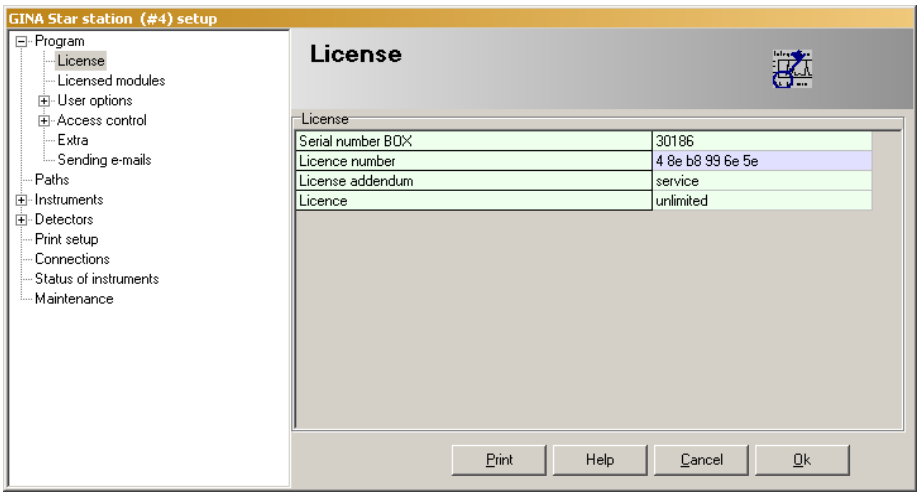


Figure 14: License

Serial number

Any license belongs to a serial number. The serial number is read out of the raytest detector or generated from your computers hardware. Don't expect the serial number of your instrument reflected here unless the communication is established. In case no serial number is obtained from the GINA Box, GINA Star generates a fixed number and indicates this by the sentence “Serial number for evaluation only”, even you may have have other data acquisition sources connected like MUCHA or Agilent GC 6850 / 6890.

License number

Type the license you got along with the software or your instrument.

License addendum

The license may be customized by some additions. The only customer relevant addition is 'profi'. This opens some rarely used special functions. These are:

Profi	This includes the run time decay correction (This is less precise than the post run decay correction), some functions in the Timed control of events
Service	Service functions are for use by raytest. These functions are described in the GINA Star software service manual.
SynthC	This converts a GINA Star demo software to a SynChrom demo software
Further	Further items may by applied to adopt customer specific needs.

License

This is the result of the license check. Normally it sais 'unlimited', which means it is not restricted to a special time period i.g. for testing.

Paths

From menu: File / Setup / Paths

See also Organization of data

Here you define how GINA - STAR stores and organizes individual data types.

Methods	Normally, you only enter a period (“.”) to save methods to the work directory. Paths starting without ‘\’ a relative to the work directory. If you change this, you have to copy existing methods manually to the new location. Enter “<user>” to use the windows user specific folder for own data.
Work directory	Select the path (drive name and subdirectory) to which your data is to be stored. Normally, this will be the hard disk 'C:\GINA_NT'. Network identifiers are allowed, i.g. \\comp\drive. Enter “<user>” to use the windows user specific folder for own data.
Archive	Select the directory in which the data are to be archived. Normally, this will be a network drive.
Always archive	Advise GINA Star to always keep the archive up to date
File structure with studies	If this item is checked, the measurement data are stored in the directory selected in the sample table
Station name contained in path	If this is checked, the data path contains the instrument name choosen in the next field
Methods for this station only	If checked, methods will be maintained in a station specific folder and are invisible to other stations.
Instruments path	The name of the instrument or station. It appears in GINA Star's title bar and the task bar. If checked, this name appears in the data path or in the method path
New file types	This is selected by default, but you may deselect this to generate files readable by GINA - DOS

Print options

Report header contains print date	The default configuration 'yes' prints the 'print' data with every report header line
Display smoothing by Savitzky Golay algorithm	The default 'yes' ('no' in GINA <=2.17) uses a less destructive smoothing algorithm for display and printout smoothing. Please notice: All filters like this generate overshoot and undershoot at signal edges. Especially negative undershoots at trailing edges (ig. signal from stable 0 to stable 5) will occur.
Company name	Enter your company name here. The company name will be printed within the report header.
Left margin in mm	The left margin for all printout
Individual page for each trace	This option allows to print a new page with the graph of one trace and the region table of this trace.
List standards in report	Prints expanded information about standards in every report.
Allow print from background process	Some printer drivers are not able to print from a background process and make unpredictable trouble, ig they block the dialog. For this reason, the feature is disabled by default and needs to be enabled on the users risk.
Print complete audit trails	This options prints audit trails with methods and runs.

Dialogs

Open File / Setup / Edit setup / User options / Dialog

Use external standards	If external standardization is licensed, you may switch this off , if it is temporarily not used.
Number of internal standards	Because an individual column is needed for each internal standard in the sample table, it helps to keep this tall, if you configure only those standards you are using.
Correct for sample	This creates a column with an entry for each sample, if the real weight is

weights	different to the nominal weight.
Calculate with dilutions	If samples are diluted, the original concentration may be obtained.
Calculate with inj. volumes	Like above
Clear sample table entries	This automatically moves entries from the sample table to the logbook.
Design with pictures	The instrument control page will use a more realistic view the the Agilent components, if enabled. Changing this will automatically position the corresponding items. Call the 'designer' just by typing <F2>. The designer always sets and positions items corresponding to the instruments in use.
Time format	Select variable to use the format 00'00 (minutes and seconds). Select minutes for minutes with 2 decimals.
Use spectrum acquisition	If licensed, you may select this to open the dialogs for spectra handling.
Instrument / Start method	The name selects the table for the graphical representation of instruments. At the same time, it selects a start method. The initial conditions from this start method are used to setup the instruments after starting GINA Star
Edit start method	Additional way to edit the start method.
Emerg method	Method to invoke for emergency situations
Preserve scaling in saved files	Scaling is always saved in files. This option establishes this scaling after opening the file.
Peak width to display limit of detection	This parameter enables a feature that helps you to estimate the necessary peak height.
Factor of sigma limit	Applies to a special report column and defines the statistical limit between secure and insecure detected peaks. See Estimate limit for more information.
Stop instruments at end of sample table	This option switches HPLC components off after the sample table is resolved. I.g. UV-lamp, pump flow, scintillator flow.

Autosampler

You may either control your autosampler by two via contacts or by a data interface. The data interface is specific to the type of the autosampler and allows to control a lot of functions within the autosampler.

Via contacts means that the start output or the start/stop outputs are connected with the respective inputs of the computer.

Autosampler	Select the type of your autosampler. If your autosampler is not in the list, please look at the menu 'extra / control subset' and select all. For details how to connect autosamplers, please see below.
Loop volume	The capacity of the injection loop
Service	Some service instructions to direct control the autosampler, i.g. 'Move needle to service position'
Volume of sample vials	The maximum volume to obtain by one draw.
Number of vials	GINA Star will check your entry in the sample table. In addition this entry helps GINA Star to find the correct representation of the autosamplers vial plate
Fixed serial number (optional 'profi')	If more than one autosampler is connected to the data interface, you select an autosampler by entering the desired serial number. This is only available, if you select the profi user option in the license addendum.

Connect autosamplers

Help is not available for all autosamplers. To see whether help is available for a dedicated autosampler, in GINA Star open File / Setup / Instruments / Autosampler, select the desired autosampler, do not confirm with Ok but push 'Help' instead.

Connections

The table shows the status of the connection to the individual instruments to be controlled. You see all information about the connection:

Output	The port. You select either a com port here or the IP address of the instrument. Please regard if you enter the IP address of an Agilent module, the address is copied to all connected modules.

Connection	The way, the instrument is connected. I.g. cable type or connection name
Function	The function of the connected instrument.
Status	If possible, some information obtained from the instrument is reflected here, i.g. the firmware version. If the connection fails, the message is displayed here.
Options / firmware	GINA Star reflects information obtained from the instruments firmare.
Serial Number	If possible, the serial number of the connected instrument is reflected here.
Instrument status	The current readyness information of the instrument is reflected here i.g. 'Lamp igniting'

Trouble shooting

Here is a list of trouble already experienced at some places and how this may probably solved.

Error message "Data behind for more than n seconds"	The data from instruments could not be transferred just in time and are lost. This may happen if the power management of the computer does not work properly. Switch off all power management and screen savers to check for this. Normally GINA Star tells the operating system not to power down during a run. See also next item 'Communication'.
Communication with Agilent components	• RS-232 Connection is too slow for spectra acquisition • Using communication via LAN without a second LAN adapter may need a switch and not a hub.
User management: no access	The user management cannot be disabled from within GINA Star, a hash is tracked instead. In case of unrecoverable trouble (I.g forgotten admin password) delete c:\gina_nt\station.glb\lRules.rDat. GINA Star. GINA Star will create an entry to the setup audit trail and allow for new settings.

Using fraction collector

The fraction collector is controlled by GINA Star with 3 functions.

Index	Move to next vial
Waste	Switch valve to collect or lead eluent to waste

Home	Move vial tray back to home position

Setup and connect the fraction collector

First open File / Setup / Edit setup / Instruments / Fraction collector and select 'Control by relay'. Then enter the number of vials available in the fraction collector. In the case you select 1100 Agilent fraction collector, you need not setup valves for connections. Instead the FC is controlled via the LAN connection.

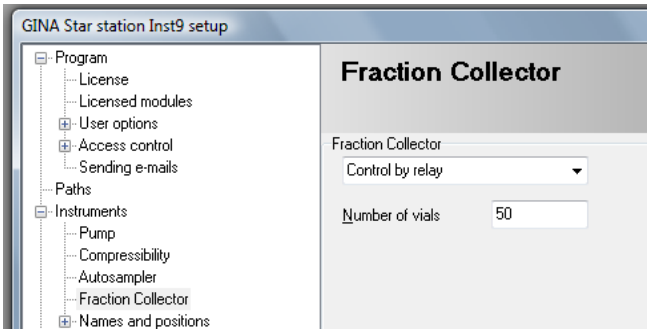


Figure 15: Enable fraction collector

Output signals

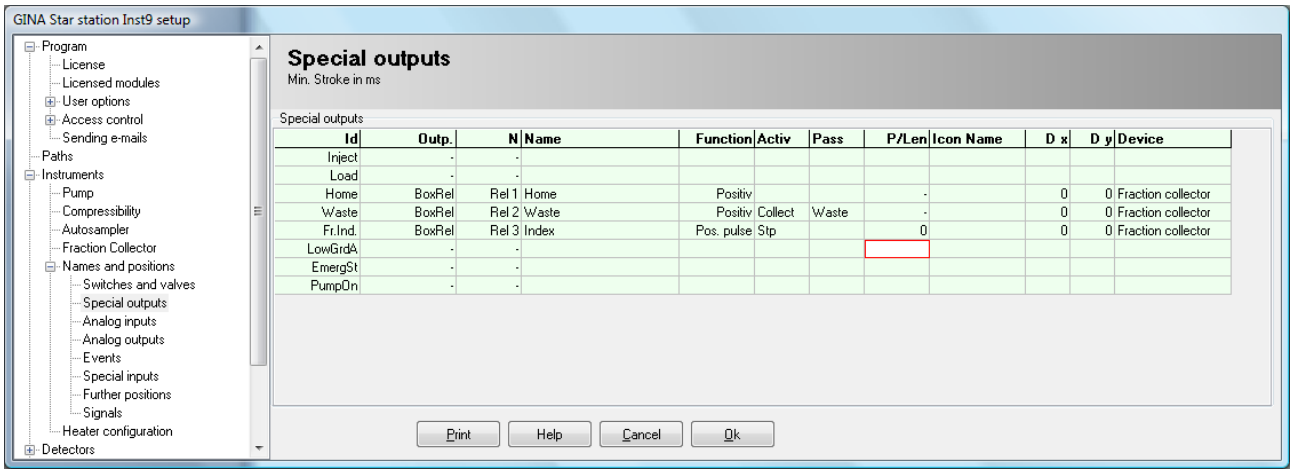


Figure 16: Output connections

The index (Fr. Ind.) signal is always available at a connector labelled 'Index'. In addition you may lead the signal to the output of your choice. Connect the indicated relais with your fraction collector.
Now you may use the fraction collector by enabling the control in your methods.

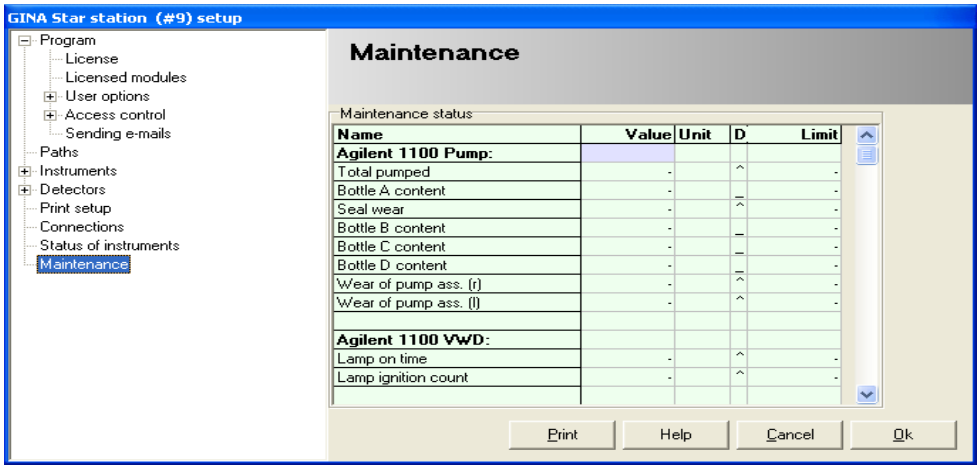
Maintenance status

File / Setup / Edit setup / Maintenance

Icon 'M' (Maintenance, optional)

The maintenance status reflects information about the age of components, which need attention from time to time or consumables to inform and remember the user about things to do. Agilent modules have especially a lot of maintenance counters. Not all of them are active. Nor will GINA Star display all activated counters. Nevertheless will GINA Star generate a message, if such counters run against the bounds.

This icon is present only if at least one instrument used in the setup does support maintenance. Unless counter values received, this icon is gray. After receiving values, this icon is either green if all counters are within the bounds, yellow, if values are near the bound or red, if the bound is exceeded. In the last case, the corresponding component will not get ready.



Picture 17: Maintenance Status

Name	The name of the counter represents the function
Value	This is the actual counter value. I.g. For the UV-lamp ignition count, the value is the number of ignitions of the lamp since last replacement. Reset this value to '0' after replacement of a lamp. You need 'maintenance' rights to accomplish this.
Unit	The unit of the counter, i.g. L for bottle contents
D (direction)	The direction informs whether the counter grows down (_) or up (^). I.g. A bottle content grows down while the lamp on time grows up.
Limit	For counters growing up, this is just the limit. Above this limit, this instrument becomes not ready. For counters growing down, limit is the maximum start value, i.g. the maximum content of a bottle.

GINA - STAR Data Acquisition Box

The data acquisition box has high resolution and highly stable integration inputs for UV-detectors, conductibility meters etc, counting inputs for radioactivity measuring systems, a control interface for analog and frequency controlled pumps and control lines with defined (start, stop, measurement active, wait for start) and free (relays, aux) specifications. At the start of the GINA - STAR program, the firmware is loaded into the BOX. This data acquisition box is available as a stand alone module as well as a part of the RAMONA Star and GABI Star.

Each box or module comes with a serial number written . This serial number is shown in GINA Star only, if a connection is established. This box has no direct setup.

ANALOG INPUTS Ch1 to Ch6	Six precision inputs with automatic null adjustment Input voltage range: -0.5 V to +4.5 V Over-voltage protection: 70 Vp-p; Resolution depending on input voltage: 4 mV to 40 mV: 10 µV 40 mV to 400 mV: 50 µV -0.5 V to 4.5 V: 150 µV Dynamic better than 1 : 500 000. The precision of the inputs requires correct connection of the measurement instruments: Please do not connect "-" signal and mass at the box side, instead have independent lines for signal "+" and "-" and one extra line for mass.
Counter inputs c1..c4	0 – 6 MHz TTL, optional 50 Ohm.
Four relay contacts	RELAY R1 to R4
STATUS SIGNALS	Meas strt and stp (Start Stop), TTL inputs (active low). Using a button, these inputs can be switched to mass to trigger the start or stop function.
STATUS SIGNAL Aux in	Input internally set to TTL level with a resistor. It can be used to limit delays during measurement.
STATUS SIGNALS Meas wt and act (measurement	TTL (active low) status outputs indicating that GINA - STAR is waiting for the start of the measurement or is currently performing a measurement, respectively.

wait and active)

STATUS SIGNAL Aux out	TTL output; this signal is used for control of waste in a sample collector. High (2.5-5V) corresponds to 'Waste'. At 'Collect' the signal goes to 0V. To use a relays contact, use GINA Star Setup / Instruments / Names and positions / Special outputs do define a relais for the waste output.
STATUS SIGNAL Frac Coll	Relay contact for control of a sample collector. This contact is closed for a period of 1 second when the sample collector should switch to the next vial.
PUMP CONTROL A, B, C (optional)	Only available when your system includes a pump control and is not provided with an interface for your pump. Frequency control: 50 Ohm outputs, range 0 Hz to 2 MHz, duty cycle 1:1 Voltages: three analog outputs 20 mV to +10 V, accuracy 0.05 % full scale, -20 mV to 1 V, 0.5 % at voltages > 1V A labeled jumper on the board is used to switch between frequency and voltage.
PUMP CONTROL Stp flw (Stop Flow)	TTL input which turns of all pumps connected to the GINA - STAR BOX between 3-15 Volt.
Serial connection	Connects the BOX via a modem cable to the computer.

GINA - STAR supports an adjustment utility. In the Setup switch pump type to 'no pump', In the menu 'radio detector' in the line 'norm count rate for analog output' type '0', <Enter> which will work immediate resulting in a should be value of –20mV at A, B and C. Adjust to –20mV by help of the corresponding 'offset' regulator. Then type ' 10020' and adjust to 10 V by help of the corresponding 'gain' regulator. The pump boards of the Ramona* have to be adjusted to –6.6 mV (instead of –20 mV) and to 3.333V (instead of 10V).

Connecting Detectors

Important notice: not regarding this important hint may result in bad and imprecise signals.

Most detectors have internal converters and provide their signals via the control interface. GINA Star uses these interfaces. To update older installations, you only have to select the appropriate signals.
Connect the integrator output of the detector with the analog input of the GINA data acquisition box. Connect the minus output of the detector with the minus input of the box and the plus output of the detector with the plus input of the box. **Important:** Connect the ground connection of the detector with the ground connection of the box. If the detector does not have a ground connection,

connect the minus output of the detector with the ground connection of the box using a separate cable.

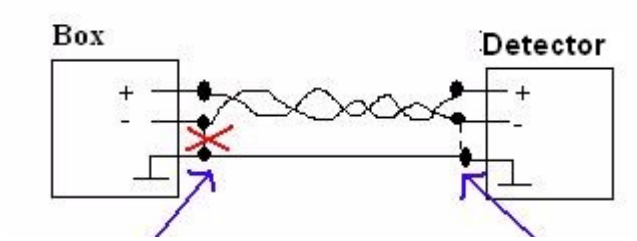


Figure 18 Connection of detector with analog output

On the GINA side, the ground must not be connected with signal-. On the detector side, you may, if no separate ground connection at the measuring instrument is available, connect the ground cable in addition to the "-" output. **If you do not observe this connection requirement, this may result in compensation circuits through one of the symmetrical and twisted conductors and thus to significant disturbances of the signals! The box measures voltages in the µV range, a cable of 2 m length easily has a resistance of 1 Ohm and the housing voltages can easily amount to several mA, i.e. exceed the sensitivity of the converters by a factor of 1000**.